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Physical and Chemical Methods of Sugar Analysis

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Physical and Chemical Methods of Sugar Analysis

A Practical and Descriptive Treatise for Use in Research, Technical, and Control Laboratories

BY

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THIRD EDITION
REWRITTEN AND RESET

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TO

DR. H. C. PRINSEN GEERLIGS

OF AMSTERDAM, NETHERLANDS

AS A TOKEN OF APPRECIATION AND ESTEEM

BY THE AUTHORS

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PREFACE TO THE THIRD EDITION

The present work is a long-delayed revision of the senior author's "Handbook of Sugar Analysis," which has now been out of print for nearly ten years. In this revision the sections of Part II of the older book on the occurrence, methods of preparation, properties, and structural relationships of the individual sugars have been omitted, since these subjects belong more properly to works dealing with the pure chemistry of the sugars and not to a book devoted primarily to sugar analysis. There have also been dropped from the present edition various obsolete copper reduction tables for the determination of glucose, fructose, invert sugar, galactose, maltose, and lactose. These omitted tables have now largely disappeared from use, and it is felt that a continuance of their publication would be of little service. A description of the methods on which these tables were based has, however, been retained for the convenience of those desiring to know the principles on which they were formulated.

The same titles and order of chapters adopted in Part I of the older edition have been followed in the present work, but all these chapters have been greatly enlarged as a result of the extensive improvements in sugar-testing apparatus and in methods of sugar analysis that have taken place since the "Handbook" was brought out in 1912. This enlargement of activity in sugar and carbohydrate analysis is reflected by developments not only in technical and control laboratories but also in research institutions. A comparison of the number of pages devoted to sugar analysis in various editions of the "Methods of the Association of Official Agricultural Chemists" indicates, for example, an increase of 40 per cent in the period between 1916 and 1935.

In the examination of sugar-containing products an increasing amount of attention is now being given to the analysis of accompanying substances which, although not belonging to the sugars, have nevertheless an important bearing on the chemical behavior of sugars in their complex technological, physical, and physiological relationships. For this reason a greater allotment of space is devoted in the present work to some of the more important of these related determinations, such as measurement of electrical conductivity, hydrogen-ion concentration, surface tension, color and turbidity, and the determination of impurities occurring in sugar products.

In the preparation of the new edition the authors have consulted the numerous articles on various phases of sugar analysis which have appeared in American and European scientific and technical journals since the publication of the first edition of the old "Handbook." In the footnote references to this extensive literature the authors have followed the system of abbreviations used by *Chemical Abstracts* in its "List of Periodicals" (126 pp. published in the index number of *Chemical Abstracts* for 1936 by the American Chemical Society, Washington, D. C.).

The amount of space in the present volume devoted to text and illustrations is over twice that in the analytical section of the first edition, the increase being largely in the final six chapters on Miscellaneous Physical Methods, Qualitative Methods, Reduction and Special Quantitative Methods, The Analysis of Sugar Mixtures, and Selected Methods for Miscellaneous Carbohydrate Products. There will no doubt be some differences of opinion as to whether in the present revision a proper balance has been maintained in the description of more recent developments in the highly complex field of sugar and carbohydrate analysis. The authors will appreciate having their attention called to possible improvements in the selection of material for future editions.

In concluding their work, which because of unavoidable delays has extended over a period of ten years, the authors desire to thank the many friends who by their suggestions and encouragement have assisted in the present revision. For the privilege of quoting methods of sugar analysis they are especially indebted to the Association of Official Agricultural Chemists, in whose collaborative work the authors have participated as referees and associate referees for many years. Special obligations are also due to Mr. R. T. Balch, of the Carbohydrate Research Division of the Bureau of Agricultural Chemistry and Engineering, for contributing the section on *p*H measurements; to Mr. Noel Deerr, of Oxford, England, for his many helpful suggestions; and to Dr. Louise McD. Browne for her valuable aid in helping to compile the index.

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WASHINGTON, D. C., and NEW YORK, N. Y.
May, 1941.

PREFACE TO THE FIRST AND SECOND EDITIONS

The subject of sugar analysis, which a generation ago was limited to determinations of density, specific rotation and reducing power, has greatly expanded within the past twenty-five years. Instruments of greater accuracy have been devised, old methods have been improved and new methods have been discovered. In the present volume the purpose of the author has been to give a rather wide, but a by no means complete, selection of the more recent methods of sugar analysis and at the same time to retain the more important features of the older textbooks.

The range of sugar analysis is so broad that in the selection of methods the author has been guided largely by his own experience in various research, technical and control laboratories. While the particular methods chosen for description may not in all cases meet with general approval it is hoped that the underlying principles of sugar analysis have been covered sufficiently to enable the chemist to make his own applications and modifications. References to special works and original articles will assist the chemist in case he desires to follow some special line of investigation more fully.

Next to the knowledge of a method the most important fact which the student of sugar analysis must acquire is the knowledge of this method's limitations. The great susceptibility of the sugars to chemical changes and to variations in specific rotation, reducing power and other " constants " is a factor which the sugar chemist must always bear in mind. The prescribed methods of analysis are usually too silent upon these points, and the inexperienced chemist often proceeds to make general use of a formula or method which has only a limited applicability. The author has endeavored to correct this tendency by including with the description of each method a brief account of its applicability and limitations.

In the examination of sugar-containing materials the problems of analysis are much simplified by a knowledge of what one may expect to find. The author has felt that a work upon sugar analysis is not complete without some description of the sugars themselves. In Part II of the present volume, he has therefore included a brief account of the occurrence, methods of preparation, properties and reactions of the different sugars and their allied derivatives. Brief

references are also made to methods of sugar synthesis; the latter play such an important part in the separation and isolation of the rarer sugars that the sugar analyst is not fully equipped without some knowledge of synthetic processes.

The principal textbooks and journals which have been consulted in preparing the present volume are named in the Bibliography. The author's obligations to these are indicated in most cases by the footnotes. In reviewing original papers, the abstracts and references contained in Lippmann's " Chemie der Zuckerarten " and his " Berichte über die wichtigsten Arbeiten aus dem Gebiete der reinen Zuckerchemie," published semiannually in " Die Deutsche Zuckerindustrie," have been of invaluable service.

In concluding his task, which has extended with many interruptions over a period of five years, the author desires to thank the many friends and coworkers who, by their help and encouragement, have greatly lightened his labors.

Special obligations are due to Dr. C. S. Hudson for reviewing the section upon mutarotation and to Prof. H. C. Sherman for suggestions upon methods for determining diastatic power. Acknowledgment is also made of courtesies extended by Mr. A. H. Bryan and by Mr. G. W. Rolfe.

For the use of cuts contained in Dr. G. L. Spencer's " Handbook for Cane Sugar Manufacturers " and in A. E. Leach's " Food Inspection and Analysis " the author owes an acknowledgment to the authors of these books and to his publishers Messrs. John Wiley & Sons. To the latter also he would express his appreciation of the hearty support which has been given and of the generous consideration which has been shown for the many delays incident to the completion of the work.

NEW YORK, N. Y., August, 1912.

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ERRATA

Page

xi, line 11. *For* INVERT OF DOUBLE *read* INVERT OR DOUBLE.

59, Table XX, third line of heading. *For* 81.49 *read* 81.12. *The last three columns of the table should read as follows:*

Volume before Mixing $E = (D + 81.12)$	Actual Volume after Mixing $F = \frac{(100 + C)}{1.0402}$	Contraction $(E - F)$
cc.	cc.	cc.
481.46	480.67	0.79
491.50	490.33	1.17
511.29	509.33	1.96
521.49	519.13	2.36

686, line 22. *For* α *read* $[\alpha]$.

724, footnote 143. *For* Bistimmung *read* Bestimmung.

912. *In the formula for the condensation product of furfural with barbituric acid there should be a* double *bond between the carbon atom at the left of the six-membered ring and the adjoining CH group.*

920. *The same correction as on page 912 should be made in the formula for the condensation product of methylfurfural and barbituric acid.*

1005, line 1 of text. *For* glucose *read* dextrin.

1005, line 2 of text. *For* (1000) *read* (1002).

1321, line 1, left-hand column. *For* Fractose *read* Fructose.

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CHAPTER I

SAMPLING OF SUGAR AND SUGAR PRODUCTS

In the analysis of sugars and sugar products, special stress must be laid upon the correctness of sample. Accuracy in analytical details is of no value unless the portion of substance weighed out for examination is an accurate sample of the entire lot of product in question. Even though the chemist is not always charged with the supervision of sampling, he should, nevertheless, acquaint himself as far as possible with the history of his product before it is received. In this way he may often explain differences which might otherwise be attributed to mistakes of analysis. A few introductory pages devoted to the general subject of sampling may, therefore, not be amiss.

The best illustration of methods of sampling, and of the errors connected therewith, is furnished by raw cane sugar. The sampling of this commodity is selected first and discussed in somewhat fuller detail.

Sampling of Raw Sugars

The raw sugar imported from the various sugar-producing countries comes in a variety of forms. Centrifugal sugar from Cuba is shipped in 325-lb. bags, that from Puerto Rico and Santo Domingo in 310-lb. bags, but Puerto Rico also uses smaller bags holding 250 lb. Raw sugar is shipped from Hawaii in 100-lb. or 105-lb. bags which are much more convenient to handle and can be re-used for packing refined sugar, after being washed, dried, branded, and lined with a cotton bag. Philippine sugars are shipped mostly in 140-lb. bags, but 100-lb. bags are also employed to some extent. The shipment of sugar in hogsheads, barrels, baskets, mats, and various other types of container that were in use before the first world war has been practically abandoned.

The need for carefully prescribed rules in sampling sugar becomes at once self-evident when we consider the different forms of the package and the exceedingly variable character of the sugar which may be contained therein. The sugar, for example, may contain lumps of higher or lower polarization than the finer part of the product; the sugar may also retain considerable amounts of molasses, which drain during transit or storage and form the " foots " at the bottom of the package. In addition to the differences in composition of sugar within the single packages

are the differences in composition between different packages of the same lot. These differences may be the result of manufacture; they may also result when no dunnage is used for covering the bottom of the holds of the ships used for transport, with the result that the bottom tiers of sugar may be damaged through absorption of bilge water. In many cases the top tiers of sugar suffer the damage, as when sugars sweat beneath the hatches; the vapors from the warm sugar rise, condense, and then drop back upon the upper layers of the cargo. If the packages of sugar run unevenly it is difficult to secure a representative fraction unless every container is sampled. The most approved method of sampling at present is to take a specimen of sugar as far as possible from every package.[1]

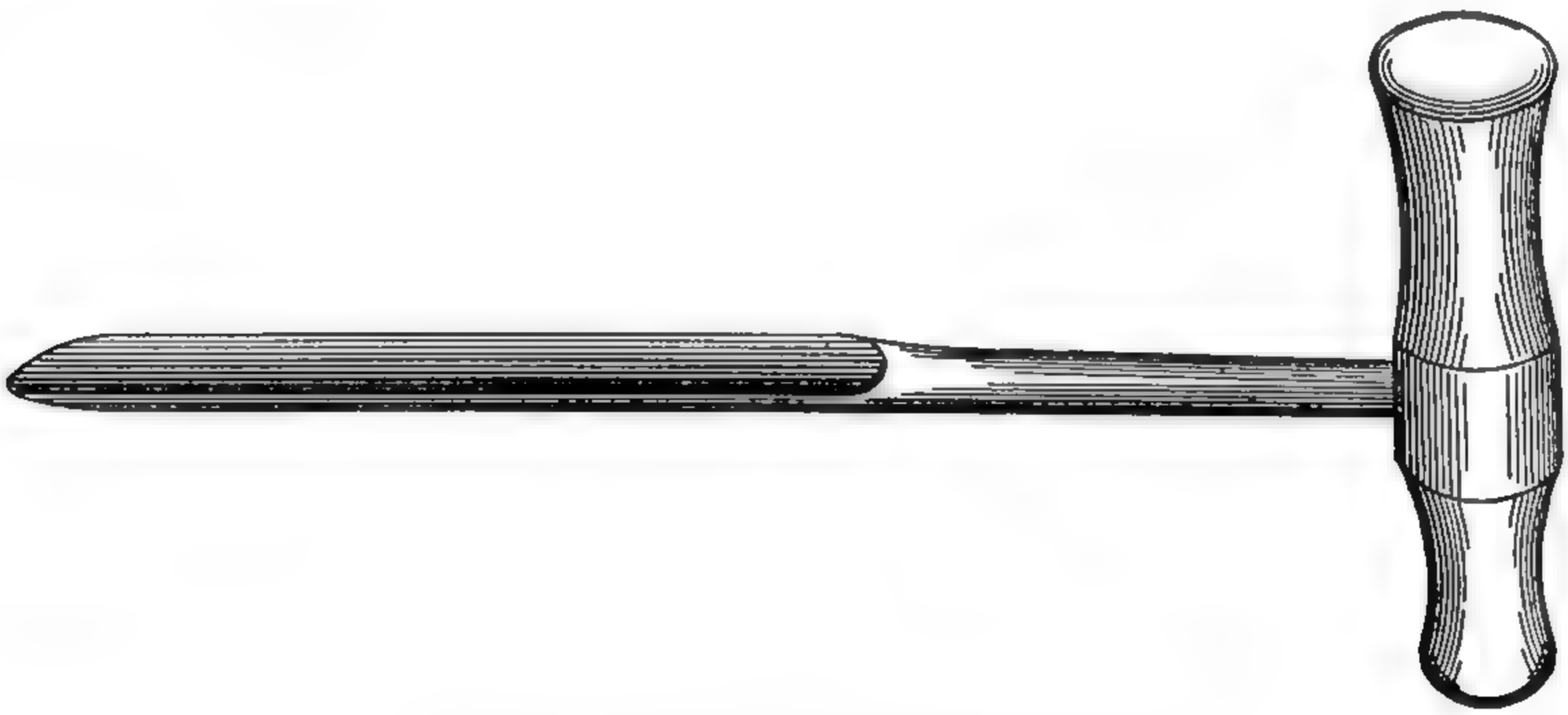


FIG. 1. Short trier for sampling sugar.

If only a certain proportion of the packages in a lot is sampled, the probable error in the polarization increases, as is shown by the following figures, given by Vondrák,[2] for a lot of 500 bags of normal raw sugar:

	PROBABLE ERROR, %
Every package sampled	±0.023
Every second package sampled	±0.033
Every fifth package sampled	±0.052
Every tenth package sampled	±0.072
Every twentieth package sampled	±0.104

Sugar is sampled in the same way as fertilizers and many other commodities — by means of a trier. This implement (Fig. 1) consists

[1] For a discussion of this and other points pertaining to methods of sampling raw sugar in different countries see paper by F. G. Wiechmann (*Intern. Sugar J.*, 9, 18–28) read before the Fifth Meeting of the International Commission for Uniform Methods of Sugar Analysis, Bern, 1906.

[2] *Z. Zuckerind. čechoslovak. Rep.*, 55, 371 (1930/31).

of a long pointed rod of steel with a groove or spoon upon one side. A thrust of the trier into the package forces the sugar along its pathway tightly into the bowl of the spoon; the sugar thus adhering, after the trier is withdrawn, is removed by the thumb, or by means of a scraper, into a covered bucket, and the process is continued until a sufficient number of packages have been sampled to constitute a mix; this number may vary, according to the size of lot and kind of sugar, from one package to several thousand.[3] The practice of the New York Sugar Trade is to mix twice daily, and in no case is a sample to remain unmixed over night.

It is of course important that the triers of the different workmen who are sampling a given lot of sugar should be exactly alike, especially as regards the dimensions of the spoons. The specifications of the United States Treasury Department Regulations[4] are very explicit upon this point and give the following dimensions of the short, long, and barrel triers.

TABLE I

DIMENSIONS OF TRIERS FOR SAMPLING SUGAR

	Short Trier	Long Trier	Barrel Trier
	cm.	cm.	cm.
Length over all	40.6	152.4	104.0
Length of spoon	22.9	132.1	91.4
Length of shank	17.8	20.3	12.7
Length of handle	26.7	38.1	30.5
Width of spoon	2.7	2.5	2.5
Depth of spoon	0.8	1.3	1.1
Diameter of handle	3.8	3.8	3.8

According to the United States Treasury Department Regulations,[5]

Sugar in hogsheads and other wooden packages shall be sampled by putting the long trier diagonally through the package from chime to chime, one trierful to constitute a sample, except in small lots, when an equal number of trierfuls shall be taken from each package to furnish the required amount of sugar necessary to make a sufficient sample. In the sampling of baskets, bags, seroons, and mats the short trier shall be used, care being exercised to have each sample represent the contents of the package.

[3] The average number of packages represented in a sample of raw sugar according to the practice of the New York Sugar Trade during recent years is about 2400.

[4] Regulations governing the weighing, taring, sampling, classification, and polarization of imported sugars and molasses. U. S. Treasury Department, Division of Customs, "Customs Regulations," 1937, Art. 721.

[5] *Op. cit.*, Art. 722.

It is necessary in sampling to keep the triers always clean; the sticking of sugar to the bowl of the spoon is especially annoying with some kinds of sugar under certain atmospheric conditions of humidity. The surface of the metal should be smooth and bright; the United States Treasury Regulations attach a penalty in case of samplers who neglect this precaution.

The general rule in sampling sugar is that the package shall be stabbed at the middle to the center, and if this practice is conscientiously followed no doubt it will give as fair a sample as can be secured under the hurried conditions of discharging a cargo. There are times, however, when it is impossible to follow this rule. Sugar which has remained for a long time in storage will sometimes solidify upon the approach of cold weather to a hard mass of material resembling concrete, a circumstance due to the evaporation of moisture and cementing together of the grain. A trier is almost useless under these conditions, and the sugar which is chipped off from the outside of the package is not a correct sample. A pickaxe is sometimes employed with hard sugar in order to open a passage for the trier; this is better than grazing the outside but is far from satisfactory. An electrically driven boring device has been proposed for sampling hard sugar.

To eliminate as far as possible the errors of personal equation in sampling, the practice of the New York Sugar Trade is for the samplers of buyer and seller to work alternately hour by hour, the one party in the interval of rest exercising a control upon the operations of the other. The tendencies to draw too high and too low from the package are thus counterbalanced and the personal errors equalized. This method seems as good as any that can be devised.

Absorption or Loss of Moisture by Samples

The liability to change in composition of the product during sampling is an exceedingly important factor in the valuation of any commodity, and more important perhaps in the case of sugar than almost any other staple. Raw cane sugar upon exposure to the air may either absorb or lose moisture according to the conditions of atmospheric humidity. If the humidity is very high or low, and the sugar is exposed to the air for any great length of time during drawing or mixing the sample, a considerable error may be introduced into the composition of the product. The buckets, which hold the samples for mixing, should always be kept tightly covered; this precaution will reduce the errors from absorption and evaporation to a large extent, although with present methods of sampling the errors from this source will never be

completely eliminated. On rainy days sugar is rarely sampled at the pier, and this is a wise precaution, considering the rapidity with which sugar absorbs moisture from a saturated atmosphere. No matter how pure the sugar, there will be absorption under such conditions, the amount of moisture taken up depending upon the initial dryness of the sugar, the fineness of the grain, and the hygroscopic character of the impurities present.

If a layer of sugar is placed in a dish over water under a closed bell jar, it will soon absorb moisture enough to liquefy, and, according to the phase rule, this absorption of moisture will continue until the pressures of water vapor for solution and atmosphere are the same. Theoretically this limit is infinity, and if the dish under the bell jar is weighed from day to day it will be found that the liquefied sugar will continue to attract moisture as long as one cares to follow the experiment.

If the atmosphere is not completely saturated, the absorption of moisture by the sugar is less rapid, and with further decrease in humidity a point of equilibrium is soon reached where there is neither absorption nor evaporation. This point of equilibrium, which represents equality of vapor pressure between the moisture of the sugar and the air, is different for different sugars. With still further decrease in humidity the sugar begins to give up moisture, the rate of loss increasing as the percentage of saturation in the air becomes less and less.

TABLE II

VARIATIONS IN MOISTURE CONTENT OF SUGARS

Kind of Sugar	Grain	Polarization	Moisture in Sugar	Gain First Hour, 100 Per Cent Humidity	Change First Hour, 60 Per Cent Humidity	Total Change at Point of Equilibrium	Humidity at Equilibrium	Residual Moisture at Equilibrium
			per cent	per cent	per cent	per cent	per cent	per cent
Granulated.....	Fine	99.85	0.10	1.78	+0.03	+0.01 (2 hours)	56	0.11
Peruvian.....	Large	98.40	0.35	1.09	−0.09	−0.14 (4 hours)	56	0.21
Puerto Rico....	Medium	96.40	1.31	1.40	−0.54	−0.73 (2 hours)	62	0.58
Philippine mats.	Fine	87.45	3.12	1.80	−0.68	−1.25 (6 hours)	56	1.87
Cuban molasses.	Large	82.75	4.85	1.12	−1.00	−2.42 (24 hours)	59	2.43

In Table II the percentages of moisture which different sugars gain or lose at 100 per cent relative humidity and at 60 per cent relative humidity are given, and the changes in moisture content at the point of equilibrium. Two grams of sugar were spread in a thin layer upon a watch glass and the change in weight noted after regular intervals of time in one case over water under a bell jar, and in the other case upon exposure to the open air. The temperature of experiments was 20° C.

After the point of equilibrium was reached upon exposure of the above sugars to the air, no change in weight was noted as long as the temperature and relative humidity remained unchanged; with fluctuations in the latter corresponding gains and losses were always observed in the weight of the sugars.

As to the absorption of moisture by sugars under excessive humidity, no relationship can be traced in Table II between composition and rate of absorption. The refined granulated sugar and the low-grade mats have equally high absorptive powers and the high-grade Peruvian crystals and the Cuban molasses sugar equally low absorptive powers. If the grain of these sugars is compared, however, it will be seen that the Peruvian crystals and molasses sugar of low absorptive power have the largest grain and that the granulated sugar and mat sugar of highest absorptive power have the smallest grain, so that the physical condition of the sugar is a very important factor in the influences which bear upon absorption.

As to the evaporation of moisture from sugars under diminished humidity, the table shows a very definite relationship between composition and rate of evaporation, this rate being, as would be supposed, roughly proportional to the initial moisture content of the sugar. The percentage of residual moisture in a sugar at the point of equilibrium is a function of the hygroscopic power of the non-sugars, and is greatest with the sugars of lowest purity (highest molasses content).

The point of greatest importance, in the bearing which these results have upon the changes in composition of sugar during sampling, is that the gain or loss in weight through absorption or evaporation of moisture is most rapid at the beginning. A comparison made by Browne in 1912 of the changes in moisture content which sugars undergo upon exposure to the air shows that the relationship between time and loss or gain in moisture follows approximately the well-known equation for slow reactions, $k = \frac{1}{t} \log \frac{a}{a - x}$, in which a is the total change in moisture content at the point of equilibrium, x the loss or gain in weight at the end of any given time t, and k the coefficient of velocity, which is a constant quantity for each kind of sugar under fixed conditions of temperature and humidity.

The assumption is frequently made by samplers of sugar that the errors from absorption and evaporation of moisture by the sample will equalize one another in the long run. This, however, is far from being true. The percentage of moisture in the ordinary grades of raw cane sugar is considerably above the equilibrium point for the average relative humidity at the port of New York. It should be stated, however,

that the loss from evaporation under the prescribed conditions of sampling is nowhere near as great as that in the above experiments, where the sugars were exposed to the open air in a thin layer. The error, however, does exist, and unless due care is exercised by the sampler there will be a very noticeable difference in the test.

The moisture-absorbing power of various sugars, polysaccharides, and sugar-containing products in an anhydrous condition is indicated in Table III from results by Browne.[6]

TABLE III

PER CENT MOISTURE ABSORBED FROM AIR AT 20° C.

Anhydrous Material	60 Per Cent Humidity 1 Hour	60 Per Cent Humidity 9 Days	100 Per Cent Humidity 25 Days
Starch	1.04	12.98	24.37
Cellulose	0.89	5.37	12.57
Agar	0.88	20.34	42.98
Maltose	0.80	6.97	18.35
Raffinose	0.74	12.90	15.91
Lactose	0.54	1.23	1.38
Molasses	0.46	9.66	68.92*
Honey	0.44	10.00	74.10*
Commercial glucose	0.29	9.00	47.14*
Malt syrup	0.28	8.84	50.96*
Fructose	0.28	0.63	73.39*
Commercial invert sugar	0.19	5.05	76.58*
Rhamnose	0.18	5.00	13.12
Pure invert sugar	0.16	3.00	73.96*
Glucose	0.07	0.07	14.50
Mannitol	0.06	0.05	0.42
Sucrose	0.04	0.03	18.35*

* Moisture absorption still progressing at end of 25 days.

It will be noted that fructose and fructose-containing materials, such as honey, molasses, and invert sugar, are exceedingly hygroscopic at high atmospheric humidities. The sampling of fructose-containing products and the preservation of samples of such products must be most carefully performed in order to prevent changes in composition as a result of moisture fluctuation.

The average moisture absorptive power of fructose for different months of the year under the climatic conditions of New York City is shown in Table IV.[6] The monthly figures for absorption represent the average increase in weight, taken at weekly periods, of a 1-g. sample exposed to the air in a weighing bottle in a dust-proof cabinet.

[6] *Ind. Eng. Chem.*, 14, 712 (1922).

TABLE IV

Absorption of Moisture from Air by Fructose for Different Months of the Year

Month	Average Room Temperature	Average Relative Humidity	Average Moisture Absorption
	°C.		per cent
January	19.5	53.9	13.7
February	19.7	47.5	11.9
March	20.4	56.7	12.8
April	20.6	58.9	13.2
May	21.9	61.8	18.3
June	25.3	71.5	21.2
July	25.7	70.0	28.4
August	24.2	72.1	24.2
September	22.2	71.4	23.2
October	19.9	69.0	22.6
November	20.0	60.1	19.2
December	19.2	56.0	13.5

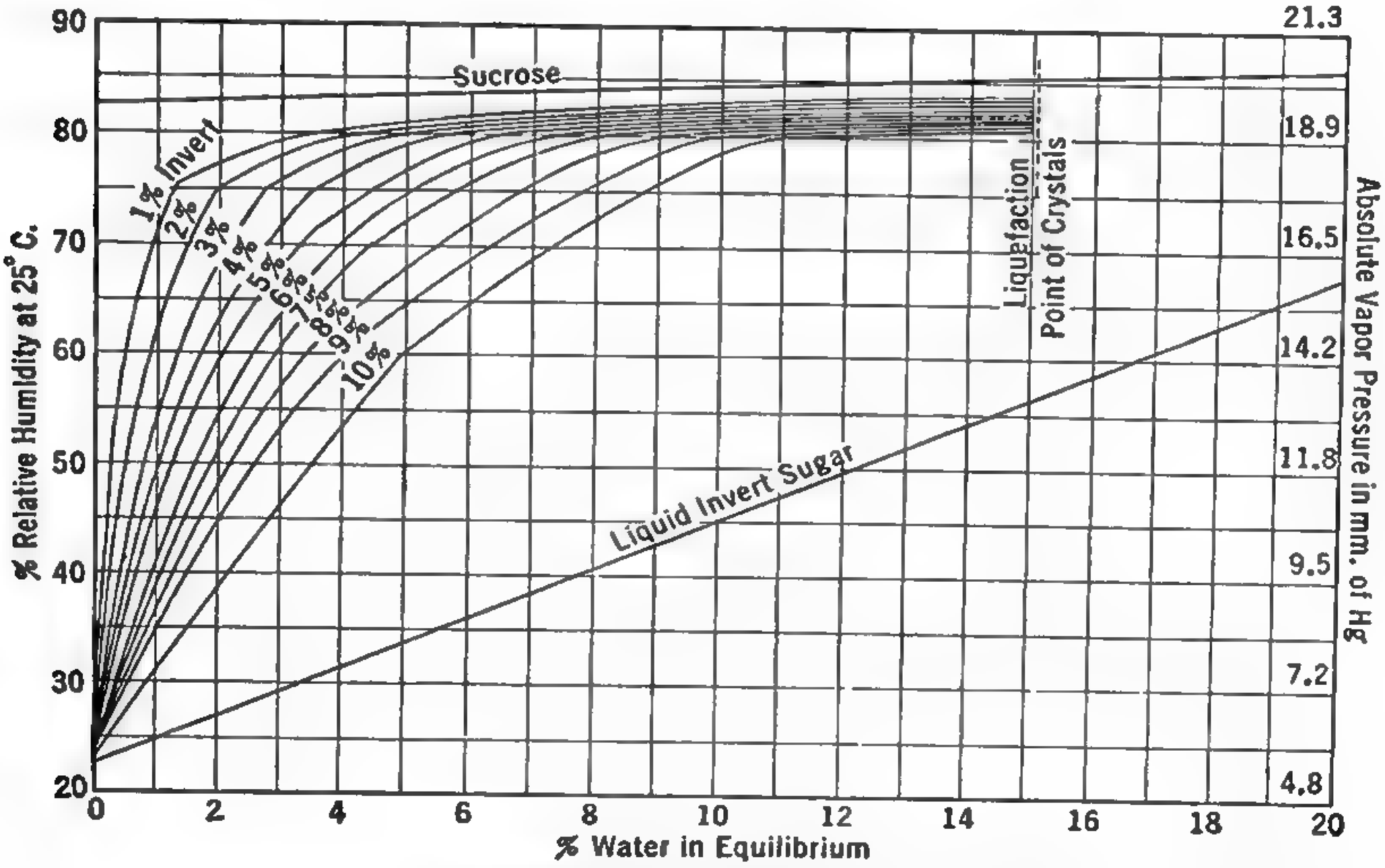
Results similar to those given above for different sugars have been reported by Sokolovsky.[7] The sequence of the sugars, according to their moisture-absorptive power, varies with the relative humidity and with the time of exposure. It also depends on the physical characteristics of the sample, such as grain size. In general, fructose is the most hygroscopic, but under certain conditions maltose absorbs more moisture than fructose. Caramel is also very hygroscopic, though less so than fructose.

According to vapor-pressure measurements on saturated solutions of four sugars, by Whittier and Gould,[8] lactose is the least hygroscopic, followed by galactose, glucose, and sucrose.

Dittmar[9] has determined the quantity of water absorbed when sucrose, glucose, fructose, and invert sugar are in equilibrium with air of varying relative humidity at 25° C. Fructose was found to be stable below 57.5 per cent relative humidity, but when the latter was increased to 67.5 per cent, the fructose took up 20 per cent of water. Crystalline glucose does not absorb water until the relative humidity reaches 80 per cent, and sucrose not below 82.5 per cent. But with both these sugars a slight increase in the humidity above these points causes absorption of considerable amounts of water. Non-crystalline fructose or invert sugar, prepared by careful heating to the melting point, are much more hygroscopic than the crystalline forms, being

[7] *Ind. Eng. Chem.*, **29**, 1422 (1937).
[8] *Ind. Eng. Chem.*, **22**, 77 (1930).
[9] *Ind. Eng. Chem.*, **27**, 333 (1935).

stable only below 22.5 per cent relative humidity. The water absorption rises rapidly with increasing humidity, and at 67.5 per cent relative humidity the amount of water taken up is the same as for crystalline fructose. The results of similar experiments on mixtures of sucrose with 1 to 10 per cent of invert sugar are shown in Fig. 2. The amount of water absorbed rises very rapidly at first, up to about 70 to 80 per cent relative humidity, and then it increases more slowly, the curves becoming parallel to the sucrose curve. Extrapolation shows that at about 20 per cent of invert sugar the curve coincides with that for non-crystalline invert sugar. Similar investigations on raw sugars are referred to on p. 21.



(Reproduced with permission from Ind. Eng. Chem., 27, 333.)

FIG. 2. Equilibrium chart for sucrose-invert sugar mixtures at 25° C.

In order to prevent changes in the composition of sugar during sampling, as a result of drying out or of absorption of atmospheric moisture, Reed[10] has devised a tightly closed sample can of firm metal containing a heavy movable grid. The cover of the can is provided with an opening for the insertion of the trier. A scraper within the opening removes the sugar adhering to the groove of the trier, and the self-closing cover of the opening moves a counting device which records each thrust of the trier and hence the number of packages sampled. When the requisite number of bags has been sampled the can is rotated on trunnions

[10] German Patent, 266454, Class 89c, Group 16, July 25, 1912.

by means of a crank, which serves to mix the sugar, any lumps that are present being reduced to fineness by the friction of the grid against the walls of the container.

The regulations of the United States Treasury Department[11] provide that, when the sample is ready to be composited, the contents of not more than three sample buckets are to be mixed on a metal table top or on a sheet of heavy glossy paper, to avoid the absorption of moisture. The sample is to be passed through a wire screen of $\frac{3}{8}$-inch mesh. The mixing must be done thoroughly, but rapidly, to prevent exposure to the atmosphere as much as possible. The cans or jars to receive the sample are compactly filled, labeled, and sealed, after which they are sent to the chemists who are to make the polarizations.

The uniform methods adopted in Czechoslovakia[12] prescribe, for mixing, the use of a metal basin, 50 to 60 cm. in diameter and 15 to 25 cm. high, and of a sieve, fitting on top of the basin, of 4- to 5-mm. mesh, and made of wire about 1 mm. thick. The sugar is placed in the sieve and passed through it into the basin by a circular motion with the hand or with a spatula. Any lumps in the sugar are broken up with the fingers. When the whole mass has passed through the sieve it is further mixed in the bowl for 2 to 3 minutes by a circular motion. This method has almost eliminated the necessity for making referee tests. But the long time necessary for the preparation of the sample may lead to a change in the moisture content.

Another occasional source of error in the sampling of sugar is the introduction into the sample of particles of bag, basket, mat, shavings of barrels, etc., from the package by the trier. The error from this cause is usually trifling; there are times, however, when it may be considerable. Such fragments of extraneous matter do not belong to the sugar, and it devolves upon the chemist to eliminate them as far as possible before weighing out the sugar for polarization. In removing foreign material from sample sugar the chemist must carefully discriminate, however, between trash which belongs to the sugar and refuse which is introduced during sampling.

Compositing and Mixing of Samples

In addition to removing trash, the chemist must complete the mixing of the sample. Lumps must be crushed and thoroughly incorporated with the rest of the sample. Even samples of sugar which are well mixed at the point of sampling must be mixed again at the laboratory owing to the segregation of foots at the bottom of the can or bottle.

[11] "Customs Regulations," 1937, Arts. 735, 736, 737.

[12] *Z. Zuckerind. čechoslovak. Rep.*, **56**, 23 (1931/32).

A neglect of such mixing of the sample in the laboratory is a cause of frequent differences between the results of different chemists. This mixing of the sample must be done with the utmost dispatch in order to avoid the errors due to absorption or evaporation already mentioned. Mixing of the sample upon paper or other porous substance which would absorb moisture is especially to be avoided. The method of mixing followed by the New York Sugar Trade Laboratory is as follows:

When samples are brought into the laboratory during freezing weather, the cans or bottles are first allowed to come to approximately the room temperature before opening and mixing. This is done to guard against condensation of moisture upon the cold sugar, which would lower the polarization. The sugar is poured out from the can upon a clean sheet of plate glass, all pieces of bagging, baskets, mats, etc., are removed, and the sample is thoroughly mixed with a clean steel spatula. Lumps are reduced by means of a steel rolling pin and incorporated with the rest of the sample. The plate glass and steel rolling pin are cleaned and wiped perfectly dry each time before using. The reduction of lumps is of greatest importance in securing uniformity of sample; the difference in polarization between the lumps and the fine portion of some sugars has been found to vary several per cent. The can from which the sugar was taken is then filled about three-fourths full, the excess of sugar upon the plate being discarded. By leaving a little empty space in the can, the weighing out of the sample by the chemist is facilitated.

Sampling of Juices, Sirups, Molasses, and Liquid Sugar Products

The sampling of juices, sirups, molasses, and other liquid sugar products involves no special difficulties provided that the material is of even composition throughout the body of the container. A large glass or metal tube may serve for withdrawing samples of molasses, etc., from the bungholes of hogsheads, barrels, and casks, when other means are not available. Containers of different capacity should be sampled separately, and in making composite samples each individual fraction should be proportionate to the total amount of material from which it was drawn.

The regulations of the United States Treasury Department[13] governing the sampling of sirups and molasses are as follows:

Molasses and Sirups — Better Grades. In sampling all grades of sirups and molasses other than blackstrap, 100 per cent of the packages shall be sampled. The contents of each receptacle shall be thoroughly stirred in

[13] "Customs Regulations," 1937, Arts. 731, 732, 733.

order that any settlings shall be evenly distributed and the contents brought to as uniform a density as possible. Receptacles of the same size shall be sampled in groups of not more than 25, a sample of uniform quantity being drawn from each. A tally shall be kept and the label thereon shall show the number of packages which each bucket represents. The dock list accompanying the sample buckets shall convey the same information and shall account for every package of the mark. Packages of different size or character of contents, although invoiced and permitted under the same mark, shall be separately sampled, tested, and classified. If any package or packages shall, in the judgment of the sampling officer, have the appearance of sirup of cane juice, or of testing by the polariscope above 50 sugar degrees, a separate sample of same shall be taken.

Blackstrap Molasses. When blackstrap (frequently designated waste molasses) is imported in barrels, 10 per cent of all the receptacles shall be sampled. When, in the judgment of the examiner or sampler in charge, a greater percentage is necessary to fairly represent the importation, such additional barrels shall be sampled as, in his judgment, are necessary to secure a representative sample. Blackstrap imported in tank vessels shall be sampled as it is being pumped from the vessel. Samples of uniform quantity, in general ½ liter, shall be drawn with such frequency as to insure one sample for each 5000 gallons.

Molasses in Tank Cars. Molasses in tank cars shall be sampled by drawing two similar complete samples of about 1 liter each and consisting of a continuous portion or core extending from the top to the bottom of the tank. When for any reason such a core cannot be obtained, the two complete samples shall each consist of three portions: One portion from the top just below the surface to the liquid, one from the center, and one from the bottom of the tank. All samples shall be forwarded to the appraiser with the least possible delay.

Automatic Juice Sampling. In sampling the juices from mills and diffusion batteries in sugar factories, various automatic sampling devices have been devised for the purpose of securing a sample of the main body of juice at each instant of time. The simplest form is the wire drip sampler in which a stout wire is arranged in an inclined position so that the upper end is in contact with the juice stream; a small portion of the juice runs along the wire into the sample container. Coombs's drip sampler (Fig. 3) is another illustration of such a device. A defect of such automatic contrivances is that they do not always give a flow of sample proportionate to the total amount of juice. The use of petcocks for diverting flowing juice into a sample bucket is not advisable with raw juice, because they clog too easily.

An automatic juice sampler,[14] originally devised at the Calumet

[14] For drawings and detailed descriptions of this device see articles by G. L. Spencer, *Ind. Eng. Chem.*, **2, 253 (1910)**; **3, 344 (1911)**.

Plantation in Louisiana, is designed to take samples in proportion to the quantity of juice produced. It consists of a metal plunger with a perforation near one end and connected to a crank, driven by the mill roll shaft or juice pump, so that a reciprocating motion pushes the plunger into the stream of liquid and withdraws the sample. The sample flows from the perforation in the plunger into a canal which empties into the storage vessel containing the preservative.

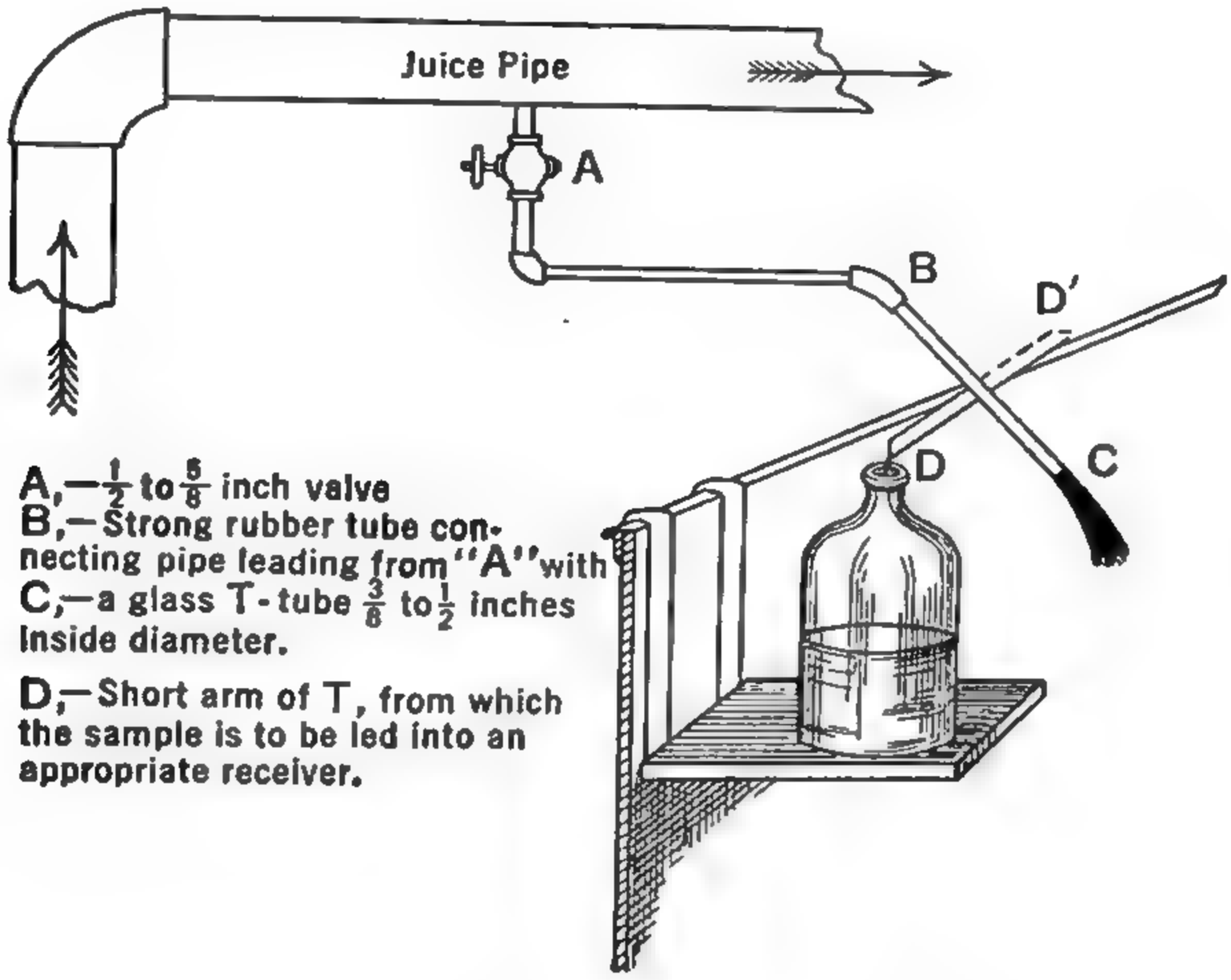


FIG. 3. Coombs's apparatus for sampling sugar juices.

Other types of mechanically driven samplers are the Westcoatt spoon sampler,[15] and the Mercedita juice sampler,[16] both actuated by the mill roller shaft, and a similar device used in Java.[17] The last takes a sample across the entire width of the juice gutter, while the other two sample at only one point. A sampler designed by Conklin[18] automatically removes a sample of juice when the discharge valve in the juice weighing tank is opened.

The juices and liquors of a sugar factory may also be sampled at the point of discharge into receiving tanks or clarifiers by the weir

[15] "Methods of Chemical Control of the Association of Hawaiian Sugar Technologists," p. 29, 1931.

[16] Spencer-Meade, "Handbook for Cane Sugar Manufacturers," 7th ed., p. 299, 1929.

[17] *Proefstation Java-Suikerind.*, Bull. 5, p. 18, 1928.

[18] "Methods of Chemical Control of the Association of Hawaiian Sugar Technologists," p. 28, 1931.

sampler of Jordan.[19] The liquid is allowed to spill over the edge of a vertically turned nipple to which a slotted weir is attached. The overflow through the slot of the weir gives a sample which is proportional to the total flow of liquid.

In grinding sugar cane, when it is desired to test the work of maceration or to determine the relative efficiency of each mill, the juices from the several sets of rollers are sampled and analyzed separately, the results of the work enabling the chemist to calculate the composition of the so-called "normal" juice or to determine the extracting power of each mill. This phase of sampling, however, belongs to the subject of sugar-house control, and the chemist is referred to the special treatises by Spencer, Prinsen Geerligs, Deerr, and others.

Errors of Sampling Due to Segregation

By segregation is meant the uneven distribution of the constituents of a substance produced by gravity, capillarity, crystallization, evaporation, hygroscopic action, occluded air, or other cause.

Segregation of Sugar. A serious error in the sampling of liquid sugar products is often occasioned by the crystallization and separation of sugar within the container. The deposition of sucrose crystals from molasses, and from maple, cane, and sorghum sirup; the granulation of strained honey by the separation of crystallized glucose; and the formation of a crust of sugar on the surface of fruit jellies are familiar examples of the phenomenon. Containers of molasses, sirup, and honey frequently have a compact layer of crystals upon the bottom. Samples taken from the liquid surface and from the crystalline deposits of such products show the greatest difference in composition. It is necessary, therefore, to mix thoroughly the contents of a container before sampling. In the laboratory the crystallized sugar in a sample of sirup, molasses, or honey should be redissolved by gentle warming before beginning the analysis. This is impracticable, however, in sampling these products in bulk from casks or hogsheads, and the most that the sampler can do is to mix the contents as well as possible by shaking and stirring.

The sampling of leaky containers, which allow the escape of liquid but retain all crystallized solids, is a fruitful cause of wide, and often puzzling, discrepancies in analytical results.

Segregation of Insoluble Non-Sugars. The composition of liquid sugar products is also affected by the settling out of insoluble salts and

[19] For detailed description and drawings see article by W. L. Jordan, *Ind. Eng. Chem.*, 13, 640 (1921).

organic non-sugars. The extent of the changes in composition which may result from this cause is shown in the following analyses by Browne of the top and bottom portions of a Cuban molasses:

	Water	Sucrose	Invert Sugar	Ash	Organic Non-Sugars
	per cent	per cent	per cent	per cent	per cent
Top of molasses.....	21.87	22.63	33.88	6.37	15.25
Bottom of molasses...	20.38	20.44	32.29	10.23	16.66

It will be noted that owing to the settling out of insoluble mineral and organic constituents, the top portions of an imperfectly mixed molasses may contain more water, sucrose, and invert sugar and the lower portions more ash and organic non-sugars.

Influences Promoting Segregation. In raw sugars, which are exposed to the air, evaporation, capillarity, and hygroscopic action tend to produce an uneven distribution of the constituents in the liquid films of residual molasses that surround the crystals of sucrose. This is illustrated by the four raw cane sugars whose analyses are given in Table V, the samples of which were kept for 1 month in tin cans closed with loosely fitting covers.

TABLE V

INFLUENCE OF SEGREGATION UPON COMPOSITION OF RAW SUGARS

	No. 1		No. 2		No. 3		No. 4	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Water.....	0.53	0.55	1.75	1.89	2.92	3.16	3.70	4.01
Sucrose.....	97.05	97.05	91.75	91.60	88.76	88.49	87.80	87.31
Invert sugar...	1.32	1.30	5.23	5.09	3.40	3.19	2.92	2.81
Ash.....	0.38	0.36	0.59	0.57	2.05	2.03	1.77	1.77
Organic non-sugars..	0.72	0.74	0.68	0.85	2.87	3.13	3.81	4.10
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Invert sugar ÷ Sucrose	0.0136	0.0134	0.0570	0.0555	0.0383	0.0360	0.0332	0.0322
Purity coefficient...	97.57	97.59	93.38	93.36	91.43	91.38	91.17	90.96

It will be noted that the sucrose, invert sugar, and ash are usually higher in the top portions of the sugars, while the water and organic

non-sugars are higher in the bottom portions. The fact that the ratio of invert sugar to sucrose (the so-called "glucose ratio") is slightly greater in the top portions would indicate that the higher invert-sugar content is not due entirely to a concentration from drying out but that there has been also a slight displacement of some of the soluble constituents in the liquid films as a result of capillarity.

With very wet sugars the drainage of liquid from the sirupy films upon the sucrose crystals to the bottom of the package or container will cause an accumulation of the soluble impurities in the lower layers of sugar. The lower layers therefore will contain less sucrose and more water, ash, invert sugar, and organic non-sugars than the upper layers of sugar. This is shown in the following analyses of the top and bottom portions of a raw sugar that had been damaged by wetting:

	Water	Sucrose	Invert Sugar	Ash	Organic Non-Sugars
	per cent	per cent	per cent	per cent	per cent
Top portion of sugar...	3.79	88.44	5.64	0.69	1.44
Bottom portion of sugar.....	5.59	84.87	6.74	0.83	1.97

The presence of occluded air in sugar products may also produce an uneven distribution of ingredients during storage as is shown in the analyses of Table VI by Fiehe[20] of the top, middle, and bottom portions of a strained honey which had undergone crystallization.

TABLE VI

INFLUENCE OF SEGREGATION UPON COMPOSITION OF STRAINED HONEY

Ingredient	I Top Crust	II Middle Portion	III Bottom Portion
	per cent	per cent	per cent
Water.....	6.60	15.70	11.25
Invert sugar..	82.75	77.08	81.75
Sucrose.....	5.00	3.20	3.37
Non-sugars....	5.65	4.02	3.63
Ash.....	0.07	0.05	0.05

The honey, which had been strained in a centrifugal machine, was highly charged with air bubbles. These, upon rising, carried along insoluble impurities, glucose crystals and other suspended particles which were left at the surface in the form of a porous crust of low water

[20] *Z. Untersuch. Lebensm.*, **55**, 64 (1928).

content. The settling of glucose crystals to the bottom of the container produced in this region a material of higher reducing sugar and lower water content than that in the middle portion.

Errors of Analysis Due to Change in Composition of Samples

Owing to the liability of sugar products to change in composition through evaporation or absorption of moisture and through decomposition by the action of enzymes or microorganisms, it is important that analyses be begun as soon as possible after samples are received. It frequently happens, however, that samples must be sent for a long distance, or stored for a considerable time, before examination can be made; the long storage of products is often necessary, as in the case of reserve samples which are retained for the purpose of confirming an original analysis in the event of doubt or dispute. The sources of error from change in composition of samples will be briefly considered.

Changes in Composition of Samples through Evaporation or Absorption of Moisture. Changes in composition due to this cause are prevented by hermetically sealing the samples in a perfectly tight container. If cans are employed all joints and connections should be soldered; cans of swaged metal, free from seams, are very desirable, but it has not been found possible as yet to manufacture these in large sizes. The covers should fit the cans closely, and the space between the two should be sealed by means of melted paraffin or by a band of adhesive tape. In many respects wide-mouth glass bottles or jars are the best containers for samples; the stoppers or corks of these should be sealed by melted paraffin or wax.

In a series of experiments by Staněk[21] upon the drying out of samples of raw beet sugar in unsealed cans, the average daily evaporation of moisture for 1 month was 0.0115 per cent; when the covers of the cans were sealed with adhesive tape (leucoplast) the average daily evaporation for 1 month was reduced to 0.0006 per cent. This loss from evaporation, of course, is not evenly distributed but is greatest during the first few days. Samples of raw cane sugar kept in covered but unsealed cans frequently show a daily increase in polarization, through loss of moisture, of 0.05 to 0.10 sugar degrees during the first days of storage.

In some experiments conducted by Browne and Hardin[22] at the New York Sugar Trade Laboratory in 1918 the changes in composition of sugar samples in unsealed tin cans were found to be as follows:

[21] *Z. Zuckerind. Böhmen*, 34, 155 (1909/10).
[22] *Louisiana Planter*, 62, 233 (1919).

	Days Stored	Original Weight	Final Weight	Loss	Per Cent Loss	Polarization		Polarization Increase
						Original	Final	
		grams	grams	grams				
Loosely packed, average 10 samples	34	396.73	395.35	1.38	0.35	96.03	96.42	0.39
Tightly packed, average 10 samples	36	445.92	444.51	1.41	0.32	96.00	96.25	0.25

In another set of experiments with very moist sugar the average daily loss in weight as a result of evaporation was found to be 0.021 per cent for unsealed cans, 0.016 per cent for cans with covers sealed with tape, and 0.012 per cent for cans with covers sealed with tape and paraffin. The loss in the last case was due to the escape of moisture through the seams of the cans.

In experiments by Šandera,[23] beet raw sugar kept in friction-top cans at 15° to 25° C. and at 65 per cent relative humidity lost only 0.0078 per cent per day. This type of can, with all seams soldered, is used officially in Czechoslovakia, in Germany, and also by the New York Sugar Trade Laboratory, which formerly employed the slip-cover cans referred to above, with crimped seams.

Glass containers closed with cork and sealed with wax are not always moisture tight owing to the formation of blow holes, produced by the escape of heated air from the pores of the cork through the melted wax. A second and third dip of the corks in the melted wax is usually necessary in order to make such containers absolutely tight. Glass fruit jars, sealed with rubber gaskets, covers, and spring clips, are also ineffective in preventing the escape of moisture, owing to irregularities of surface between cover and jar and imperfections in the rubber seal. The most convenient and effective sample container for sugars and sugar products is a bottle with ground glass stopper, sealed with melted wax or paraffin. The unsealed stopper does not prevent the evaporation of moisture.

Changes in Composition of Samples through Action of Enzymes. Changes in composition due to this cause are frequently noted during the storage of plant substances, such as grains, seeds, fruits, and tubers. The change may consist in an inversion of sucrose by action of invertase, in a conversion of starch by action of diastase, in a modification of gums, hemicelluloses, etc., by action of other enzymes, or in a loss of sugars through respiration. It is impossible to preserve untreated

[23] *Z. Zuckerind. čechoslovak. Rep.*, **51**, 309 (1926/27).

plant materials of the above description for any length of time without change in composition, although the rate of change may be greatly retarded by cold storage. Heating the samples before storing will destroy enzymes but has the disadvantage in some cases of causing inversion or of liquefying and saccharifying starch. Freezing the material may suspend enzyme action for the time, but may on the other hand incite changes of a different sort, as in the production of sucrose from starch in frozen potatoes.

When samples of fresh plant materials, which are liable to undergo enzymic decomposition, cannot be analyzed immediately, an effective method of preventing change is to weigh out a quantity of the finely reduced substance and preserve in a stoppered jar or bottle by the addition of alcohol. An excess of alcohol (over 50 per cent) destroys the action of enzymes, and samples thus preserved do not undergo any change in composition after many months' standing.

Changes in composition through enzyme action may also occur in cold-strained honey. A case is known where a bottle of such honey, which contained over 20 per cent sucrose at the time of sampling, contained after 4 months' storage less than 10 per cent; in a second sample of the same honey, which was kept in a warm laboratory during the same period, the sucrose was almost completely inverted. The inversion was probably due to an invertase secreted by the bees. The action of enzymes in such products as honey may be destroyed by heating the sample to a temperature of 80° C.

Changes in Composition of Samples through Action of Microorganisms. The effect of yeasts, molds, and bacteria in changing the composition of sugar products is well known. While the conditions for the development of microorganisms are most favorable in such dilute media as juices and musts, they may also cause deterioration in such concentrated products as molasses and sugar. The fermentation of such a thick menstruum as molasses, however, is confined entirely to the surface, which, through the attraction of hygroscopic moisture, becomes dilute enough to favor microorganic growth. The same is true of raw sugars; the film of molasses coating the crystals undergoes a gradual fermentation, with the result that the underlying sucrose is slowly dissolved and inverted.

The changes which may occur as a result of fermentation in stored samples of raw cane sugar may be seen from the following polarizations made by Browne[24] at the Louisiana Sugar Experiment Station upon several samples of Cuban Centrifugal sugars after having been kept 9 months in the can.

[24] *La. Agr. Expt. Station Bull.* 91, 103 (1907).

TABLE VII

DETERIORATION OF SUGAR SAMPLES IN STORAGE

Number	April, 1904	January, 1905	Decrease
	Polarization	Polarization	
1	96.50	95.60	0.90
2	96.05	95.00	1.05
3	95.50	93.20	2.30
4	94.20	91.70	2.50
5	97.15	94.60	2.55
6	93.95	91.10	2.85
7	94.70	91.20	3.50
8	95.00	91.20	3.80
9	95.90	91.50	4.40
10	96.80	90.70	6.10
11	96.20	89.00	7.20
Average	95.63	92.25	3.38

The change in composition of stored samples of raw cane sugar, as a result of inversion by microorganisms, is shown by periodic analyses of Browne[25] in Table VIII.

TABLE VIII

PERIODIC ANALYSES OF BAD-KEEPING SUGARS

Date of Analysis	Sample	Polarization	Water (W)	Sucrose (S) by Clerget	Invert Sugar	Ash	Undetermined	$\frac{W}{100-S}$
			per cent	per cent	per cent	per cent	per cent	
May, 1915...	A	96.15	1.25	96.39	0.91	0.47	0.98	0.346
	B	94.85	1.65	95.18	1.22	0.62	1.33	0.343
	C	95.85	1.18	96.34	1.13	0.56	0.79	0.322
	D	95.55	1.31	95.82	0.86	0.70	1.31	0.313
	Average	95.60	1.35	95.93	1.03	0.59	1.10	0.331
October, 1915.	A	95.55	1.27	95.75	1.61	0.45	0.92	0.298
	B	94.35	1.65	94.70	2.01	0.61	1.03	0.311
	C	95.15	1.09	95.54	2.04	0.54	0.79	0.244
	D	94.65	1.41	95.09	1.83	0.65	1.02	0.287
	Average	94.93	1.35	95.27	1.87	0.56	0.95	0.285
January, 1916.	A	95.55	1.34	95.92	1.64	0.46	0.64	0.328
	B	94.10	1.55	94.65	2.12	0.61	1.07	0.289
	C	94.95	1.12	95.59	2.02	0.55	0.72	0.254
	D	94.55	1.39	95.10	1.90	0.67	0.94	0.284
	Average	94.79	1.35	95.31	1.92	0.57	0.85	0.288
August, 1917.	A	94.30	1.40	94.61	2.30	0.43	1.26	0.260
	B	93.10	1.58	93.72	2.61	0.67	1.42	0.252
	C	93.85	1.23	94.80	2.65	0.57	0.75	0.237
	D	93.05	1.59	94.06	2.69	0.68	0.98	0.268
	Average	93.58	1.45	94 30	2.56	0.59	1.10	0.254

[25] *Ind. Eng. Chem.*, **10**, 178 (1918); see also *Louisiana Planter*, **54**, 281 (1915).

The analyses show that during the warmer months of the year there is a loss of sucrose and a corresponding gain in invert sugar. The ratio W/(100 — S), the so-called " factor of safety " of the Colonial Sugar Refining Company of Australia, is the index of the keeping quality of a sugar. It was found by Browne that sugars whose factors of safety exceeded 0.31 deteriorated badly while those whose factors were below 0.29 suffered no appreciable change in composition.

If the direct polarization is used instead of the sucrose content in making the calculation, the safety factor of 0.29 becomes 0.26. The New York Coffee and Sugar Exchange has adopted 0.25 as the marginal value for safe keeping. The factor of safety does not apply to washed sugars, or to sugars which have already undergone partial deterioration.

Thieme[26] has determined, for Java raw sugars, the relative humidity at which the vapor tension of a sugar of a given safety factor is in equilibrium with the atmosphere. Sugars with a safety factor of 0.25, based on polarization, are stable at 65.3 per cent relative humidity; above that point they take up moisture, below it they dry out. For a safety factor of 0.20 the corresponding relative humidity is 61.7 per cent. Rendon[27] gives 66.2 per cent relative humidity as the safe maximum for the storage of Philippine raw sugars; this indicates a safety factor of 0.265 according to Thieme. Spengler and Böttger[28] recommend storage of German white sugars at a relative humidity of 60 per cent, and of raw beet sugars at 50 to 55 per cent. Experiments by Keller[29] have shown that at a relative humidity of 50 per cent white and other high-grade sugars will neither gain nor lose an appreciable amount of moisture.

The preservation of sugars and sugar products against micro-organisms by sterilization is not always desirable on account of the changes which the high temperature may produce in the physical and chemical properties of the sample. Sterilization of sugar products in order to be effective must be repeated upon several successive days owing to the extreme resistance of many spores to a single heating.

The preservation of liquid sugar products, such as juices, musts, and sirups, is sometimes effected by the addition of chemical preservatives. In the proper choice of these the chemist must be guided by the analyses that are to be performed. A 40 per cent solution of formaldehyde, at the rate of 0.5 to 1 ml. per liter of liquid, may be used, but it is not very effective, and it is excluded when reducing sugars are to be determined.

[26] *Arch. Suikerind.*, 42, I, 157 (1934).
[27] *Philippine Agr.*, 19, 383 (1930).
[28] *Z. Ver. deut. Zucker-Ind.*, 80, 690 (1930).
[29] *Sug. J.*, 2, No. 6, 25 (1939).

Mercuric chloride is more reliable; about 0.004 to 0.2 per cent will retard fermentation, but larger quantities are required for complete sterilization. Mercuric iodide, dissolved in a solution of potassium iodide, is sometimes preferred. Neutral or basic acetate of lead is a good preservative for juices and sirups to be polarized, and the former may be used also if reducing sugars are to be determined.

The preservation of succulent plant substances, such as pulp of fruits, is best accomplished by treating a weighed portion of the sample with alcohol in a stoppered jar or bottle, in the manner previously described.

TABLE IX

ALTERATION IN COMPOSITION OF CUBAN MOLASSES AS A RESULT OF SPONTANEOUS CHEMICAL CHANGES

Time of Analysis	Molasses 1				Molasses 2			
	Polarization	Sucrose	Invert Sugar	Total Sugars as Invert	Polarization	Sucrose	Invert Sugar	Total Sugars as Invert
April, 1914....	+24.86	31.30	19.10	52.04	+25.52	34.79	25.09	61.71
October, 1914..	+23.54	30.73	18.74	51.09	+21.67	32.97	26.67	61.37
May, 1915....	+22.33	30.00	18.19	49.77	+18.59	30.39	26.71	58.70
April, 1916....	+21.34	29.74	18.84	50.14	+14.63	28.38	30.19	60.08
Sept., 1917....	+19.80	28.45	19.93	49.88	+ 8.25	23.65	32.25	57.14
August, 1918..	+18.26	25.81	20.66	47.83	+ 5.72	21.39	33.27	55.79
August, 1921..	.....	24.93	20.93	47.17	.....	18.49	33.77	53.23
January, 1923.	.....	25.13	21.07	47.52	.....	16.64	34.88	52.40
July, 1927.....	+11.84	20.98	21.00	43.08	− 5.20	12.97	34.43	48.08
March, 1928...	.....	18.94	21.50	41.44	.....	11.02	35.60	47.20
March, 1935...	+ 6.4	12.61	23.57	36.84	− 9.60	6.61	34.13	41.08
Total change..	−18.46	−18.69	+ 4.47	−15.20	−35.12	−28.18	+ 9.04	−20.63

Modern freezing technique offers interesting possibilities for the preservation of sugar products. Dymond[30] has found that cane juice can be kept for several days and even longer, without deterioration, by rapid cooling to — 3° C. or lower, but the samples must be analyzed immediately after thawing.

Changes in Composition of Samples through Internal Chemical Reactions. Samples of sugar products may also undergo changes in composition as a result of internal chemical reactions, such as reduction and oxidation. The analyses by Browne[31] in Table IX show the progressive deterioration of two samples of Cuban cane molasses during 21 years of storage. The changes took place spontaneously as a result

[30] *Proc. Tenth Annual Congress, South African Sugar Tech. Assoc.*, p. 70 (1936).
[31] *Proc. 5th Congr. Intern. Soc. Sugar Cane Technologists*, Brisbane, 1935, p. 217.

of reactions between unstable organic substances and the sugars. Microorganisms were absent.

Changes of this nature, which are due to the conversion of the sugars into dark-colored huminlike substances of high carbon content, are greatly accelerated with increase of temperature. In the so-called " hot room " or " froth " fermentation, the decomposition may take place rapidly with great violence. Sudden changes of this kind have also been reported with Egyptian sugar-cane molasses stored in pits.[32]

Other essentials pertaining to the sampling of sugar-containing materials will be described in Chapter IX.

[32] " Behavior of the Molasses of the Sucreries d'Egypte," P. Neuville, *Proc. 6th Cong. Intern. Soc. Sugar Cane Technologists*, 1938.

CHAPTER II

DETERMINATION OF MOISTURE IN SUGARS AND SUGAR PRODUCTS BY METHODS OF DRYING

The accurate determination of moisture, in some respects the most simple of analytical operations, is frequently one of the most difficult determinations that the sugar chemist is called upon to make. Among the chief difficulties that confront the chemist in determining the moisture content of sugar products by the ordinary methods of drying, may be mentioned: (1) the very hygroscopic nature of many sugar-containing materials and the retention of water by absorption or occlusion; (2) the extreme sensitiveness of some sugars, notably fructose, to decomposition at temperatures between 80° and 100° C., with splitting off of water and other volatile products; (3) the liability of many impure sugar-containing substances upon heating to give off various volatile products, such as alcohols, aldehydes, esters, organic acids, carbon dioxide, and ammonia, which are wrongly estimated as water in determining the loss of weight during drying.[1] Oxidation may also occur in certain cases with formation of volatile decomposition products. The moisture determination is further complicated by the fact that many sugars, as maltose, lactose, and raffinose, retain variable amounts of water of crystallization under different conditions of drying, so that the chemist is not always certain — even when no further loss of weight occurs in the oven — as to the exact amount of moisture that may be retained in a hydrated form.

In the following description of processes for determining moisture, methods will be given for a number of typical substances. The first class of methods to be described is intended only for products that are stable at 100° to 110° C. The determination of moisture in cane sugar is taken as an illustration.

Determination of Moisture in Cane Sugar

Refined sugar, raw beet sugar, and the superior grades of raw cane sugar are dehy rated successfully by drying 2 to 5 g. of the finely powdered sample in a thin layer for 2 to 3 hours in a boiling-water oven

[1] Gustavson and Pierce (*Ind. Eng. Chem.*, 16, 167 [1924]) show that sulfur dioxide, carbon dioxide, ammonia, iodoform-producing substances, and an unidentified oil are given off by beet molasses in drying at 105° C.

and then heating in a special oven for 1 hour at 105° to 110° C. The sugar is cooled in a desiccator, and, after the loss in weight is determined, reheated at 105° to 110° C. for another hour. The process is continued until successive heatings cause no further loss.

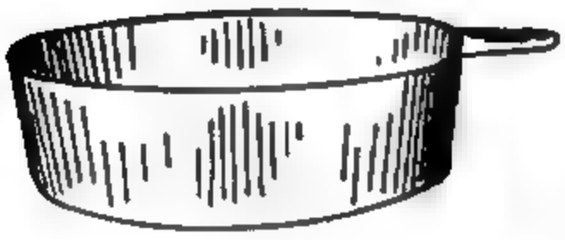


FIG. 4.

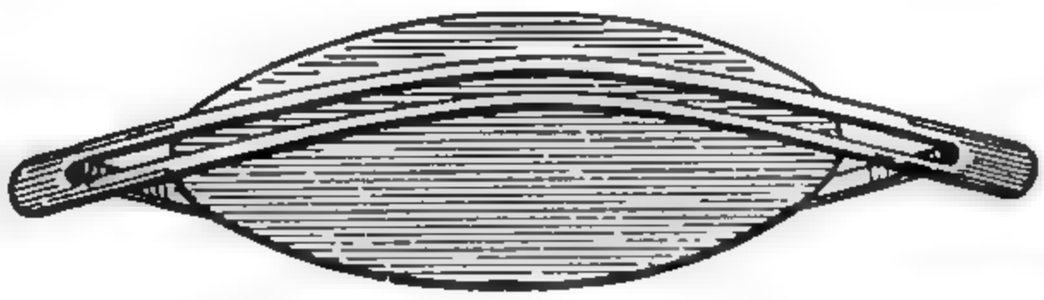


FIG. 5.

FIG. 6.

Receptacles for drying sugar.

For weighing out the sugar, flat-bottomed aluminum, nickel, or platinum dishes may be used; clipped watch glasses are also convenient. (See Figs. 4 and 5.) With lower-grade sugars, which contain hygroscopic salts and other impurities, the dish should be covered during weighing. For many purposes of dehydration low glass-stoppered weighing bottles (Fig. 6) are well suited; they prevent loss of moisture in weighing out the sample, and absorption of moisture in weighing the dry residue. Metal dishes with well-fitting slip covers may also be used.

The former official method[2] of the Association of Official Agricultural Chemists, still widely used for determining moisture in sugars, prescribes drying in a boiling-water oven for 10 hours. (See p. 38.) With some sugars, more especially those of large grain, there is danger of occlusion and retention of water, and the last traces of moisture are expelled only at 105° to 110° C.

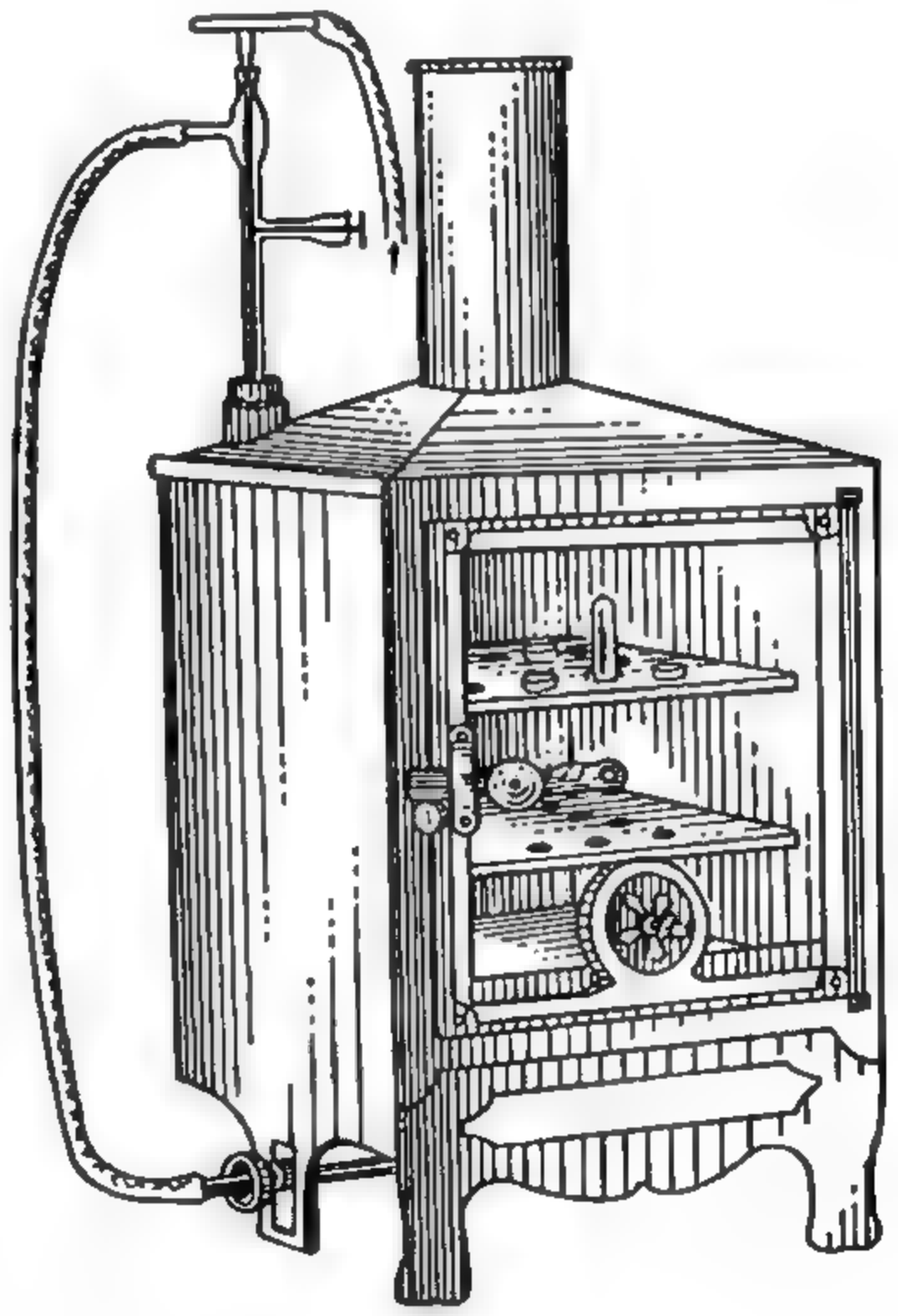


FIG. 7. Wiesnegg hot-air oven with Reichert gas regulator.

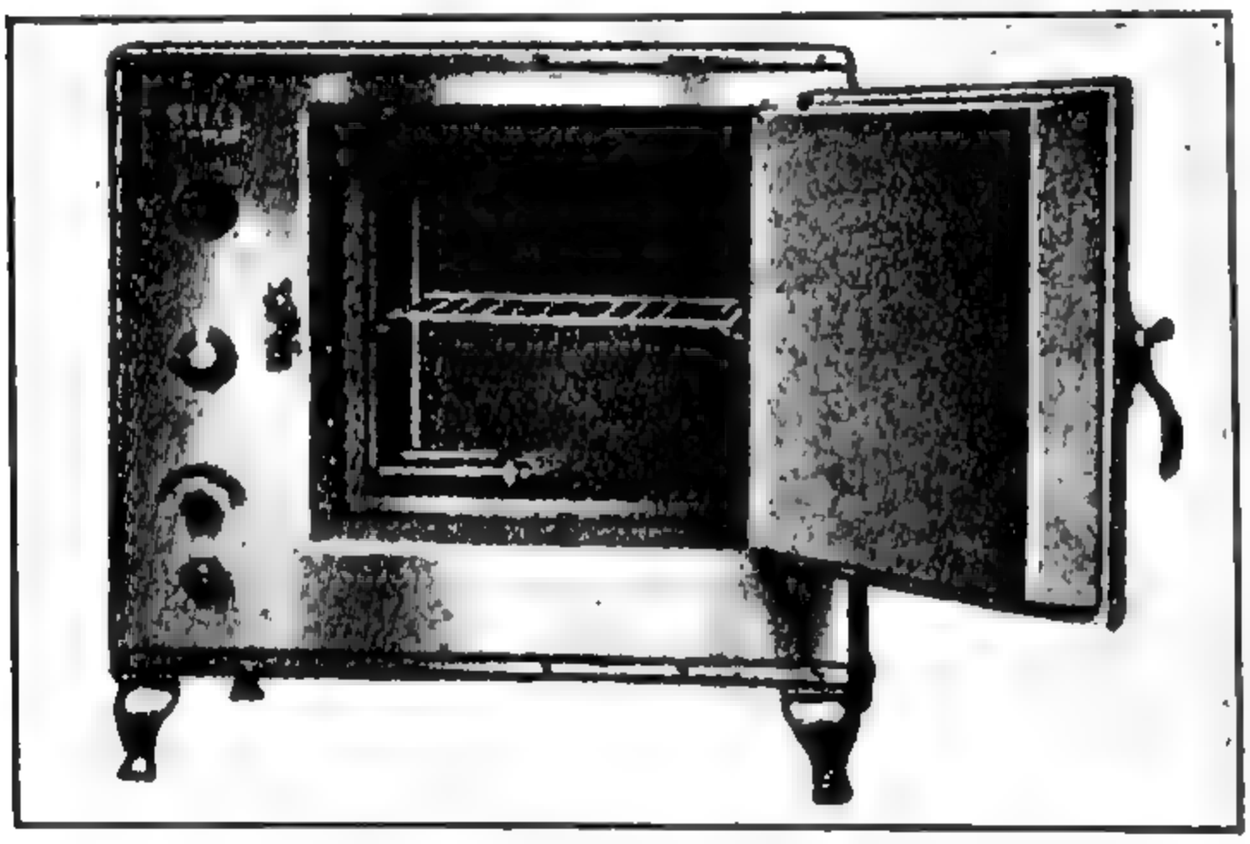
For maintaining a uniform temperature of 105° to 110° C. glycerin or salt is usually added to water in the requisite proportion to produce a liquid of the desired boiling point for use in the oven. A glycerin-

[2] "Methods of Analysis, A. O. A. C.," 4th ed., p. 462, 1935.

water mixture of the desired boiling point is less liable to corrode the metal of the oven than a salt solution and is preferred for this reason.

In case a gas-heated hot-air oven is used for drying at 105° to 110° C., the temperature should be governed by means of a gas regulator. A Wiesnegg hot-air oven with porcelain inner chamber and glass door is a very suitable type. Such an oven with a Reichert gas regulator is shown in Fig. 7. In using hot-air ovens, where considerable variations in temperature are likely to occur through unequal distribution of heat, the exact temperature of drying should be determined by a thermometer placed near the material under examination.

Freas's Electric Drying Oven. Of the various electrically heated ovens the apparatus of Freas, Fig. 8, is one of the most widely used. The outside walls are of rust-resistant iron, coated with aluminum. The walls and other metal parts inside of the oven are of stainless steel. The space between the walls, 3 inches thick, is filled with an efficient insulating material. The electric heating element is mounted at the bottom of the drying chamber, and a thick, perforated metal plate is placed over it to distribute the heat uniformly. The air enters through the perforated floor of the oven and passes out through adjustable shutters at the top. The temperature is controlled by a thermostatic regulator, placed inside of the oven so that it cannot be blocked off from the free flow of heat, but the contact points are in a sealed case outside of the oven to prevent ignition of inflammable vapors. The desired temperature is set by means of a knob and pointer on the front wall of the oven. A pilot light indicates whether the current is on or off. As soon as the walls of the oven have become uniformly heated and radiation has become constant, the temperature within the oven is controlled over a range from 35° to 150° C. with a variation not exceeding 0.5°. As the temperature is not exactly the same in all parts of the drying chamber a comparison thermometer should be placed upon the shelf near the place where the substance is being dried.

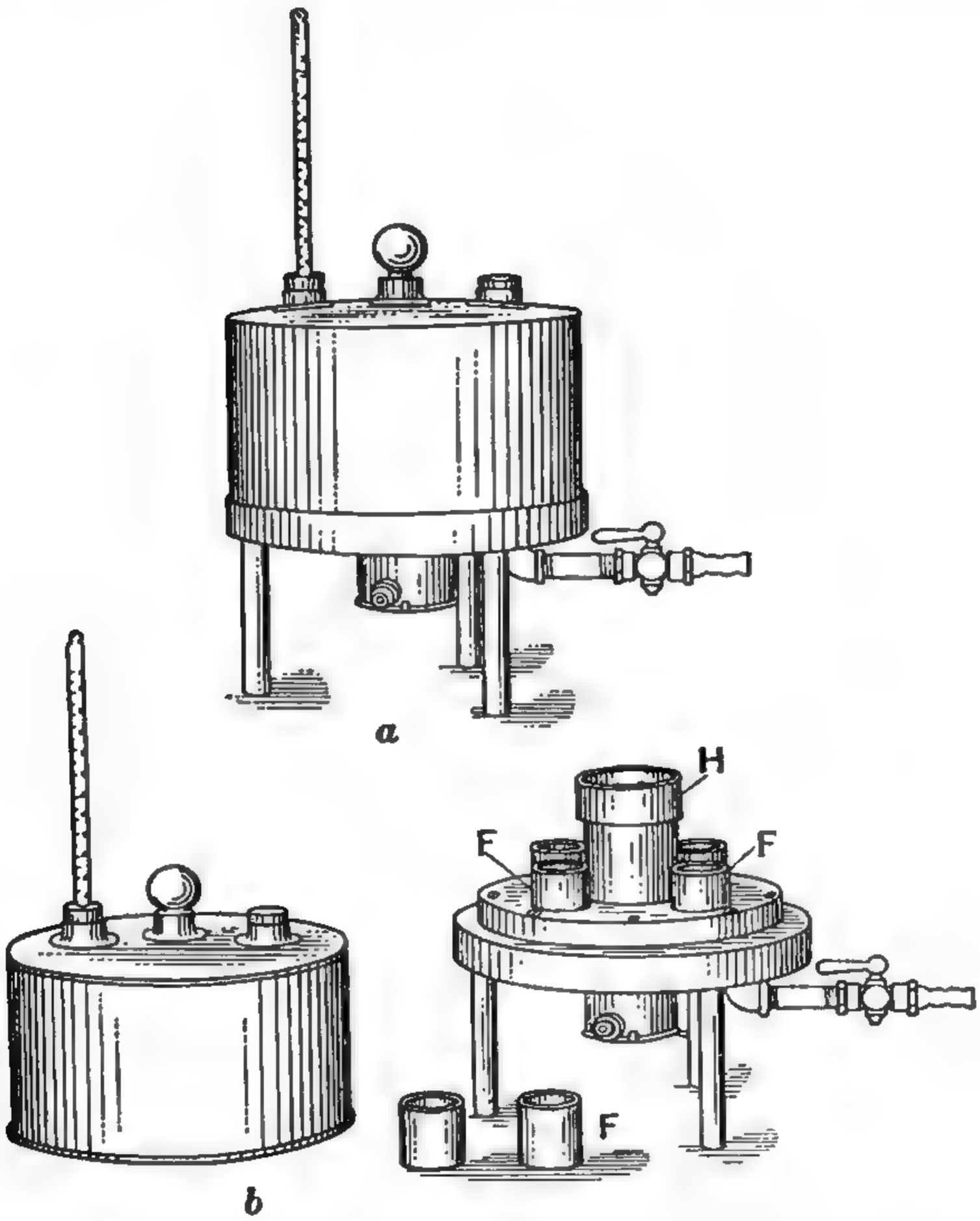


(Courtesy of Eimer & Amend.)

FIG. 8. Freas's electric drying oven.

Spencer's Electric Oven for Rapid Moisture Determination. In sugar-factory control, where determinations of moisture in raw sugars,

bagasse, press cake, etc., are often desired with the greatest attainable speed, Spencer's electric oven[3] for rapid moisture tests is used to great advantage. This apparatus with cover removed and with cover in place is shown in Fig. 9. When the cover is in place a rapid current of air, heated electrically by coils of resistance wire in the central cylinder *H*, is drawn by suction through perforated capsules *F*, *F*, which



(*Courtesy of Arthur H. Thomas Co.*)

FIG. 9. Spencer's electric drying oven.

a, with cover in place; *b*, with cover removed.

contain the product to be dried. The thermometer in the drying chamber is kept at the desired temperature (105° C. for sugar, 130° C. for bagasse) by means of a rheostat regulator. The moisture can be completely removed by this device from raw sugar in 10 minutes and from bagasse in 30 minutes without decomposition of product. A clock which operates an electric time switch and bell is placed in the circuit

[3] *Ind. Eng. Chem.*, 13, 70 (1921).

and indicates the completion of the drying period. Comparisons of determinations by Spencer's rapid electric oven with those obtained by the slower methods of drying show a close agreement of results.

Determination of Moisture in Sirups, Molasses, Massecuites, etc., when Fructose Is Absent or Present only in Traces

For dehydrating sirups, molasses, massecuites, and other sugar-containing substances, which contain but little or no fructose, the method of drying previously described may be used. The material, however, should first be absorbed upon dry sand, pumice stone, or asbestos in order to facilitate the removal of the large excess of water. The following official methods[4] of the Association of Official Agricultural Chemists are recommended for drying the semiliquid products of this class:

Drying upon Pumice Stone. Prepare pumice stone of two grades of fineness, one of which will pass through a 1 mm. sieve, the other through a 6 mm. but not a 1 mm. sieve. Digest each with dilute sulfuric acid (1 + 4) for 8 hours on a steam bath. Wash free from acid and heat to dull redness. Make the determination in a flat metallic dish 60 mm. in diameter. Place a layer of the fine pumice stone, 3 mm. in thickness, on the bottom of the dish, then a layer of the coarse pumice stone, 6–10 mm. in thickness; dry; and weigh. Dilute the sample with a weighed portion of water so that the diluted material shall contain 20–30 per cent of solid matter. Weigh into the dish, prepared as described above, the quantity of diluted sample to yield, approximately, 1 g. of dry matter. If this weighing cannot be made rapidly, use a weighing bottle provided with a cork through which a pipette passes. Dry at the temperature of boiling water, making trial weighings at intervals of 2 hours toward the end of the drying period until the change in weight does not exceed 2 mg. Report the percentage loss in weight as moisture.

Drying upon Quartz Sand. Digest pure quartz sand that will pass a 40-mesh but not a 60-mesh sieve with hydrochloric acid, wash free from acid, dry, and ignite. Preserve in a stoppered bottle. Place 25–30 g. of the prepared sand and a short stirring rod in a dish approximately 55 mm. in diameter and 40 mm. in depth, fitted with a cover. Dry thoroughly, cover dish, cool in a desiccator, and weigh immediately. Then add sufficient diluted sample of known weight to yield approximately 1 g. of dry matter and mix thoroughly with the sand. Heat on a steam bath for 15–20 minutes, stirring at intervals of 2–3 minutes, or until the mass becomes too stiff to manipulate readily. Dry in an oven at the temperature of boiling water for 8–10 hours, cool in a desiccator and weigh. Repeat the heating and

[4] "Methods of Analysis, A. O. A. C.," 5th ed., p. 484, 1940; J. Assoc. Official Agr. Chem. 23, 88 (1940).

weighing until the loss in 1 hour does not exceed 2 mg. Report the loss in weight as moisture.

Dry sand, as well as the dried sample, will absorb an appreciable quantity of moisture on standing over most desiccating agents, so all weighings should be made as quickly as possible after cooling in the desiccator.

(Aikin[5] has found that the percentages of moisture in beet-sugar factory products, as determined by the quartz sand method of drying, become greater with decreasing fineness of the absorbent material and that uniform results are obtained only with sand grains which are smaller than 0.25-mm. diameter. The method proposed by Aikin is as follows:

(Use only sand that will pass a screen with 0.25 mm. perforations; digest the sand in hot hydrochloric acid, wash, and ignite. Use 25 to 30 g. of sand, and dry and weigh just previous to making the determination. Weigh into the dish not over 1 g. of dry substance, add 1 ml. of water, place the dish on top of a drying oven until warm, mix for 3 minutes, and warm and mix again, until a perfectly homogeneous mixture is obtained. Dry at a temperature of 105° C. for 6 hours, cool and weigh. Repeat until the loss in weight after heating for a period of 1 hour is less than 0.10 per cent.

(Make all weighings as soon as the temperature of the desiccator is within 2° C. of the temperature of the balance. Repeat all determinations where the duplicates do not check within 0.20 per cent.)

Pellet's Method of Determining Moisture.[6] In a method of drying considerably employed in France, Pellet nickel capsules, 85 mm. wide and 20 mm. deep, are used. The capsule has a circular depression in the center as shown in Fig. 10. Each capsule is provided with a cover having a small notch at the edge for the passage of a small stirring rod.

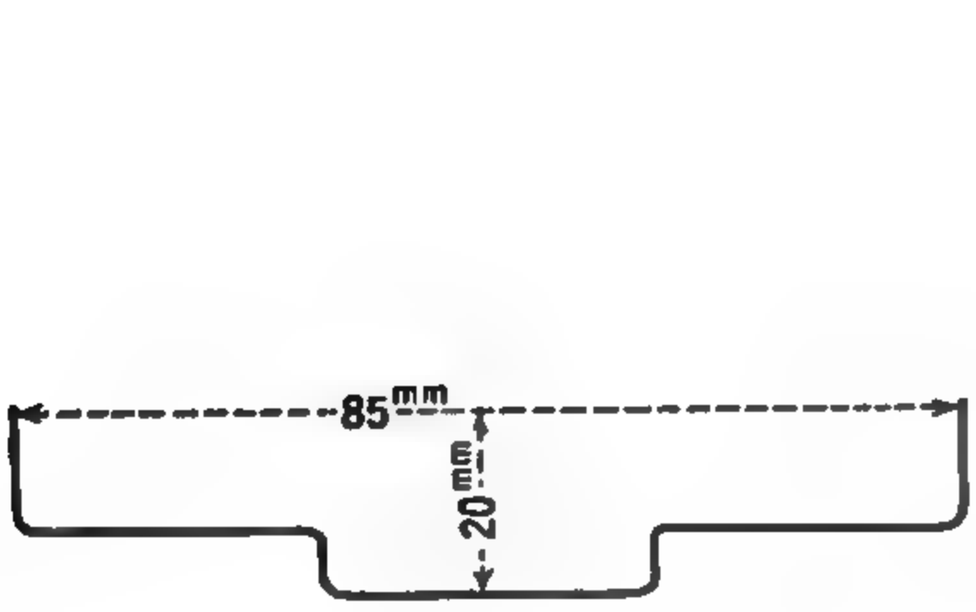


FIG. 10.

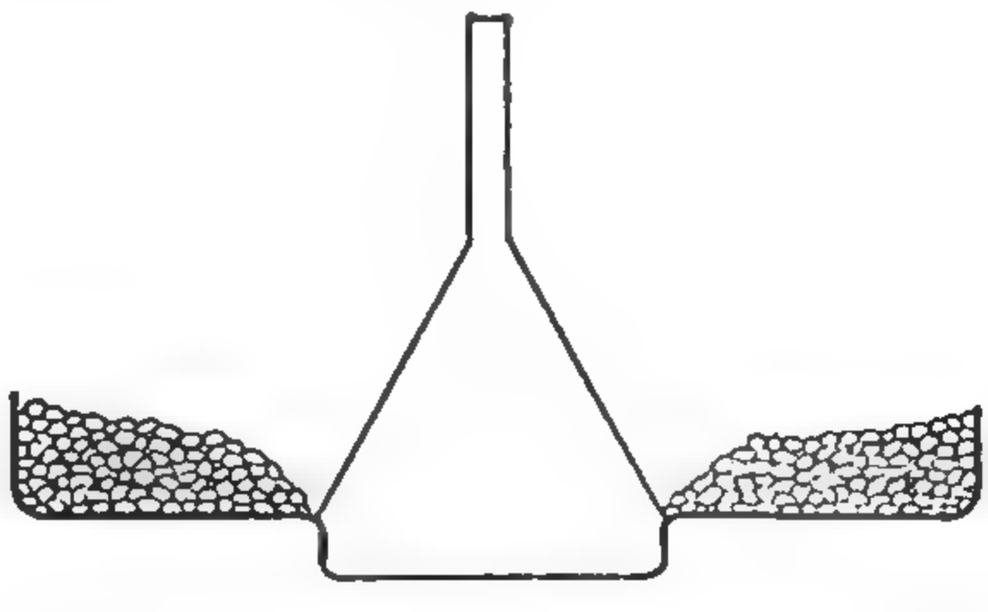


FIG. 11.

Pellet capsule for drying liquid sugar products.

The raised border of the capsule is filled with fine particles (about 1-mm. diameter) of freshly ignited pumice stone, employing an inverted funnel as shown in Fig. 11. The funnel is then removed, the cover and

[5] *Ind. Eng. Chem.*, 12, 981 (1920).

[6] Fribourg's "Analyse chimique," pp. 90–94, 1907.

stirring rod put in place, and the capsule weighed. Three grams of the substance to be dried is then weighed in the central depression of the capsule; 5 ml. of hot distilled water is then added, and after stirring to dissolve all soluble matter, the capsule is slightly inclined on different sides to permit absorption of the solution by the pumice stone. The process is repeated with 3 ml. more of hot water and then with 2 ml. The contents of the capsule are then spread evenly over the entire bottom and dried in any suitable oven at a final temperature of 102° to 105° C.

In case of products containing even traces of free acid, a drop or two of strong ammonia is added. The excess of ammonia is expelled, and the amount retained in the combined form is usually too small to be regarded. If the free acid is not neutralized, inversion of sucrose may result, with the introduction of a considerable error in the determination.

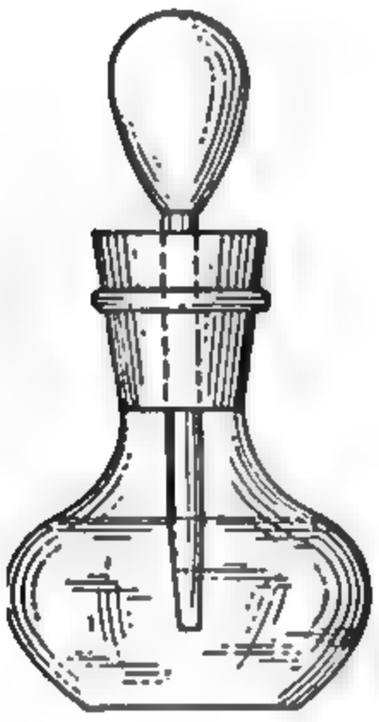


FIG. 12. Bottle for weighing sugar solutions.

In the weighing out of juices, sirups, sugar solutions, etc., for absorption upon pumice stone, sand, or asbestos, a small flask provided with a stopper and a rubber-bulbed pipette or medicine dropper will be found convenient (Fig. 12). The bottle is filled about two-thirds full with the sugar solution, which should not contain over 25 per cent solids, and then closed with the stopper and pipette. After the bottle and contents have been weighed, about 5 ml. of liquid is conveyed by means of the bulb pipette to the absorbent material, and the flask restoppered and weighed. The difference in weight is the amount of sample taken. Honeys, molasses, jellies, and other water-soluble substances of high density should be diluted before this method is employed, by dissolving a weighed amount of substance in a weighed amount of distilled water.

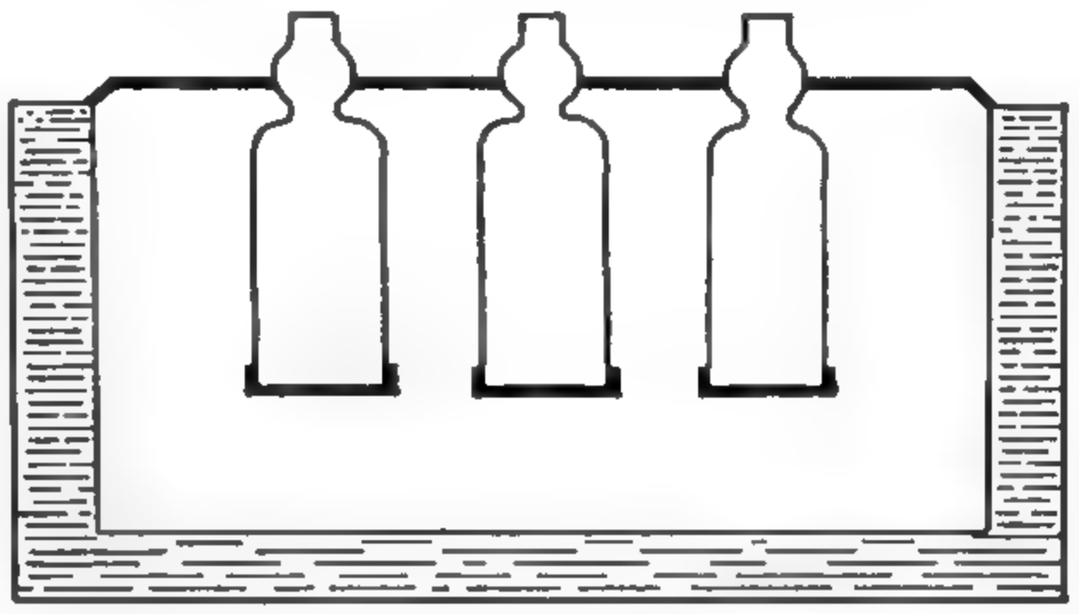
The above method of weighing samples is precluded, however, when insoluble matter is present, as with jams, sauces, and similar products. In such cases a weighed amount of the well-mixed sample is stirred with a little distilled water until all soluble matter is dissolved and then completely transferred to the absorbent material in the drying dish with the help of a fine jet of water. The Pellet method of drying is especially convenient for products of this class.

Josse's Drying Method. In Java and some other countries filter paper is used as absorbing material, as proposed by Josse.[7] A strip of filter paper, 1 cm. wide and 100 cm. long, is folded back and forth every 2 to 3 mm., zigzag fashion. It is then placed on a flat strip of

[7] *Bull. assoc. chim. sucr. dist.*, **10, 656** (1892/93).

the same width, the two strips are rolled up together, and the ends fastened with a pin. The large surface of this " rosette," with numerous air spaces, facilitates the removal of water vapor. The rosette is first dried to constant weight at 102–105° C., in a weighing dish. It is then removed from the dish; 2 to 3 g. of molasses or sirup is weighed into the dish and diluted with about 5 ml. of water. The rosette is replaced in the dish and quickly absorbs the liquid which is then dried to constant weight in the oven at 102° to 105° C.

Morizot's Method of Drying in a Current of Air.[8] In this method the time necessary for drying is appreciably shortened by passing a current of hot air through the sample. The apparatus used, Fig. 13, consists of a double-walled oven of sheet copper, 25 cm. long, 15.5 cm. wide, and 13 cm. high. The space between the two walls is filled with oil. The oven is heated either by gas, with a gas regulator, or by electric heating coils with thermostatic control. The top plate of the oven has six circular openings, in two rows of three each, with two-piece covers. The glass drying tubes have a cylindrical body, 3 cm. in inside diameter and 5 cm. long, open at the bottom and with a bulb on top. The lower opening can be closed with an aluminum cap. The upper ends of the tubes are connected with an aspirator pump by means of rubber tubing and stopcocks attached to a manifold so that each tube can be used for drying independently of the others. The cylindrical part of the tube is filled with about 6 to 7 g. of absorbent cotton which is tamped down so that the surface is about 1.5 cm. from the top of the cylinder. The tube is dried to constant weight at 105° to 110° C., being weighed with the aluminum cap in place. The sample of molasses, massecuite, etc., is first diluted with a known amount of water, a quantity of solution containing about 2 g. of solids is run onto the cotton, and the tube is reweighed. The cap is removed, the tube placed in the oven which has previously been heated to 105° to 110° C., and connected with the aspirator. The liquid is quickly absorbed by the cotton and distributed throughout. The current of air must be rapid enough to create a vacuum of several centimeters. If too much liquid has been introduced and it rises into the bulb, a new analysis must be started with a smaller quantity. At the end of the drying period the



(*Reproduced with permission from Bull. assoc. chim., 52, 830.*)

FIG. 13. Morizot's drying apparatus.

[8] *Bull. assoc. chim.*, **52**, 830, 833 (1935).

aluminum cap is replaced and the tube weighed. Constant weight is usually obtained in 1½ to 2½ hours, whereas drying on pumice stone by Pellet's method requires 5 to 6 hours. The results of the two methods check within 0.2 per cent.

Determination of Moisture in Products Which Contain Fructose

Owing to the susceptibility of fructose to decomposition in the presence of water at temperatures much above 70° C., the methods previously described are not applicable to the determination of moisture in such products as honey, sugar-cane molasses, jams, fruit products, and similar substances. The error which may result from this source may be seen from the following experiment by Carr and Sanborn upon dehydrating a solution containing 17.75 per cent of fructose. The solution was dried upon pumice stone in flat-bottomed dishes at 100° C. in air.

Hours of Drying	Percentage of Solids
1	19.02
2	18.53
3	18.57
4	18.16
5	17.42
6	17.34
8	16.90

It is seen that the percentage of solids after 5 hours' drying is lower than the actual amount of fructose taken.

Methods of Drying in Vacuum. The susceptibility of many sugar products to decomposition at 100° C. in the air induced Scheibler in 1876 to propose drying in vacuum. Weisberg[9] in 1894, and Carr and Sanborn[10] in 1895, further emphasized the necessity of vacuum drying; and at present dehydration at low temperature under reduced atmospheric pressure is the only recognized method for the accurate determination of moisture in fructose-containing materials.

Carr and Sanborn's Method. Many methods have been devised for drying sugar solutions in vacuum. The following process is the one described by Carr and Sanborn,[10] who have employed their method successfully upon the widest range of materials, such as fructose solutions, honey, molasses, sorghum, and maize juice.

Select clean, fine-grained pumice stone and divide into fragments the size of No. 4 shot. Pass the dust through a 40-mesh sieve and treat separately from the larger particles. Digest hot with 2 per cent sulfuric acid and wash

[9] *Bull. assoc. chim. sucr. dist.*, 11, 524 (1893/94).
[10] *Bull.* 47, U. S. Bureau of Chem., pp. 134–151.

until the last trace of acid disappears from the wash water. Owing to the ready subsidence of the material, the washing may be accomplished rapidly by decantation. After complete washing, place the material, wet, in a Hessian crucible, and bring to redness in a monitor or other convenient furnace. When complete expulsion of water is assured, place, hot, in a desiccator, or direct into the drying dishes if desired for use immediately. In loading the dishes place a thin layer of the dust over the bottom of the dish to prevent contact of the material to be dried with the metal; over this layer place the larger particles, nearly filling the dish. If the stone has been well washed with the acid, no harm may result from placing the dish and stone over the flame for a moment before placing in the desiccator preparatory to weighing.

If the material to be dried is dense, dilute until the specific gravity is in the neighborhood of 1.08 by dissolving a weighed quantity in a weighed quantity of water. (Alcohol may be substituted in material not precipitable thereby.) Of this, 2 to 3 g. may be distributed over the stone in a dish, the area of which is in the neighborhood of 3 sq. in., or 1 g. for each square inch of area. Distribute this material uniformly over the stone by means of a pipette weighing bottle (weighing directly upon the stone will not answer), ascertaining the weight taken by difference.

Place the dishes in a vacuum oven, in which may be maintained a pressure of not more than 5 in. mercury, absolute. The form of oven is not material so long as the moisture escapes freely by passing a slow current of air (dried) beneath the shelf supporting the dishes. The temperature must be maintained at 70° C. and the vacuum at 25 in.

All weighings must be taken when the dish is covered by a ground plate, and the open dish must not be exposed to the air longer than absolutely necessary. Weighings should be made at intervals of 2 or 3 hours.

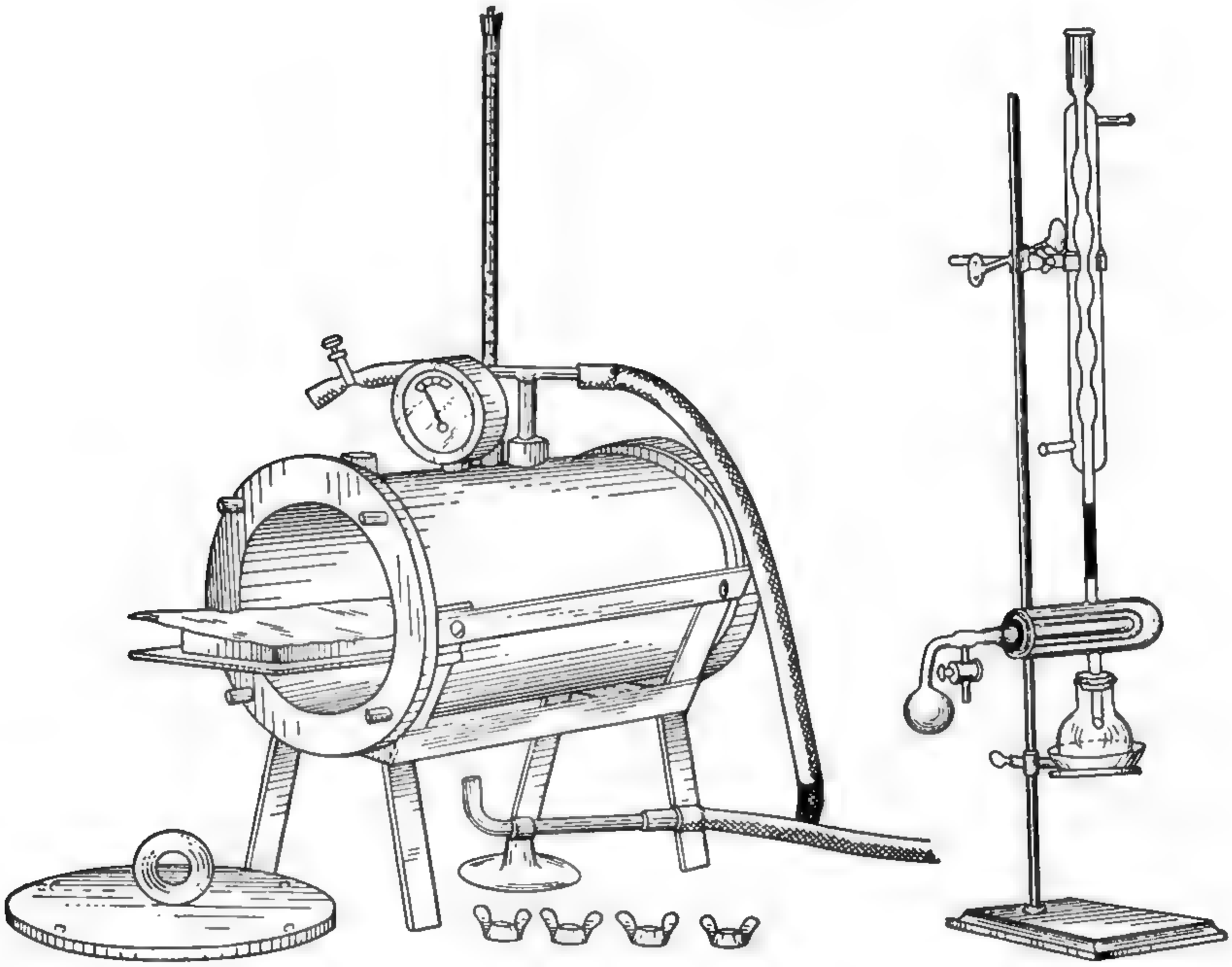
The following triplicate series of experiments were made by Carr and Sanborn upon a solution containing 17.10 per cent fructose. The solution was dried on pumice stone in flat-bottomed dishes at 70° C. under a vacuum of 25 in.

[Hours	Number 1	Number 2	Number 3	Means
	per cent	per cent	per cent	per cent
4	17.12	17.09	17.06	17.09
8	17.11	17.09	17.08	17.09
12	17.06	17.05	17.06	17.06
17	17.09	17.07	17.07	17.08

It is seen that constancy in weight is secured after 4 hours, and that no further appreciable loss takes place even after 17 hours' drying.

The Carr vacuum oven is illustrated in Fig. 14. The oven is provided with openings for attachment of manometer, insertion of thermometer, and for inlet and exit of air. A gas drier containing concen-

trated sulfuric acid may be used for removing moisture from the slow current of entering air. The detachable plate at the end of the oven is provided with a rubber gasket and is fastened into position by four screws which secure a perfectly air-tight joint. Later enlarged modifications of the Carr oven provide several tiers of shelves and dispense with the screws, the door being held in place by the suction. Liquids of constant boiling point may be used for maintaining the desired temperature within the oven.



(*Courtesy of Eimer & Amend.*)

FIG. 14. Carr vacuum oven.

FIG. 15. Abderhalden's vacuum drying apparatus.

Abderhalden's Vacuum Drying Apparatus. For drying sugar, sugar derivatives, and sugar-containing products in a vacuum under easily controllable conditions the improved drying apparatus of Abderhalden (Fig. 15) is very useful, especially for products containing water of crystallization which is removed only with some difficulty. The apparatus consists of an inner drying tube with ground-glass opening adapted to receive the curved tubular stem of a bulb containing phosphorus pentoxide. A stopcock attached to the stem of the bulb

permits the exhaustion of air from the apparatus. The drying tube, containing the dish of substance to be desiccated, is inserted through the opening of a stopper into a horizontal jacket which permits the entrance of vapors from a flask of boiling liquid below and the exit of vapors into a condenser above. By the use of selected liquids of different boiling points, such as ethyl alcohol at 78.5° C., water at 100° C., toluene at 110.5° C., *m*-xylene at 139° C., the drying tube can be maintained uniformly at the desired temperature for an indefinite period without fear that the heat will exceed the prescribed limit. The drying tubes are made of various sizes according to the needs of the analyst.

Since the selection of a liquid of the desired boiling point may at times offer some difficulties, Clark[11] has modified the Abderhalden apparatus

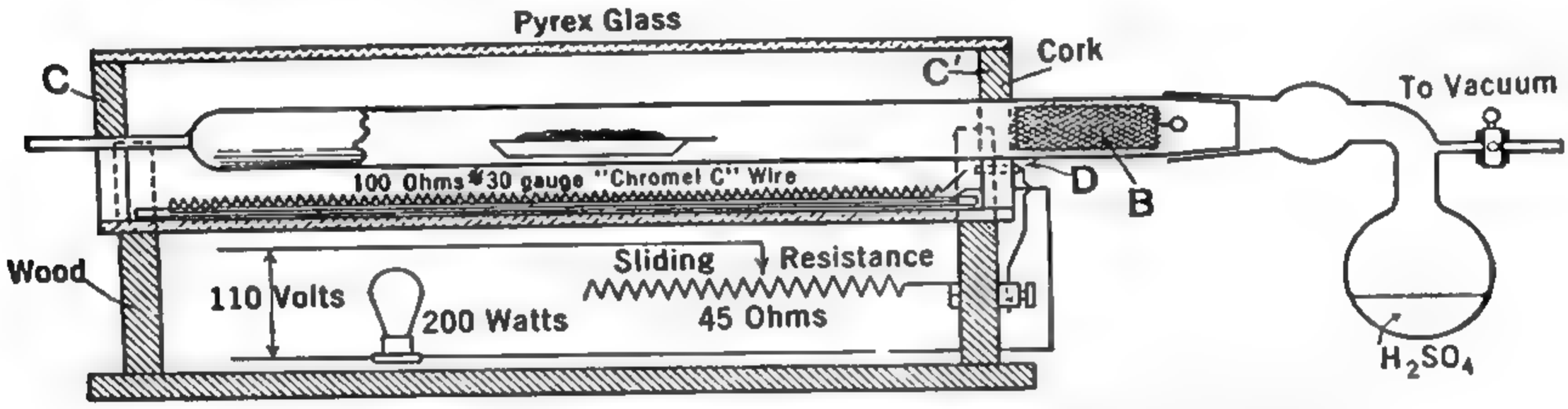


FIG. 16. Clark's modification of Abderhalden's drying apparatus.

so as to secure a more flexible range of temperatures. The modified drier (Fig. 16) consists of an outer cylindrical mantle of heavy Pyrex glass closed at the end with perforated corks, *C*, *C'*; which hold the inner drying tube in place. An electrically heated coil of No. 30 gauge "Chromel C" wire, attached to an asbestos base, rests upon the bottom of the mantle under the whole length of the drying tube which can be maintained at any desired temperature by means of a rheostat. Anhydrous potassium hydroxide, contained in a wire-gauze basket, *B*, in the cool part of the tube, removes moisture and acid vapors, while the concentrated sulfuric acid in the bulb also acts as a desiccating agent, absorbing at the same time any alkaline and organic vapors that may be evolved. This modification, in addition to other advantages, eliminates the danger of fire hazards from boiling organic liquids and the inconvenience of the condenser, which are involved in the use of the original Abderhalden apparatus.

Freas's Electric Vacuum Oven. The Freas electric drying oven, Fig. 8, is easily converted into a vacuum oven by removal of the shelves

[11] *Ind. Eng. Chem.*, **20**, 306 (1928).

and the insertion of a heavy bronze vacuum cylinder. This cylinder is attached on both sides to metal tubing, for which closable openings are provided in the walls of the oven, for manometer connection and for exhaustion and intake of air. The front of the vacuum cylinder is closed with a cast-bronze cover which makes an air-tight joint without the use of rubber gaskets or screws. The vacuum chamber is provided with shelves for the support of the drying dishes.

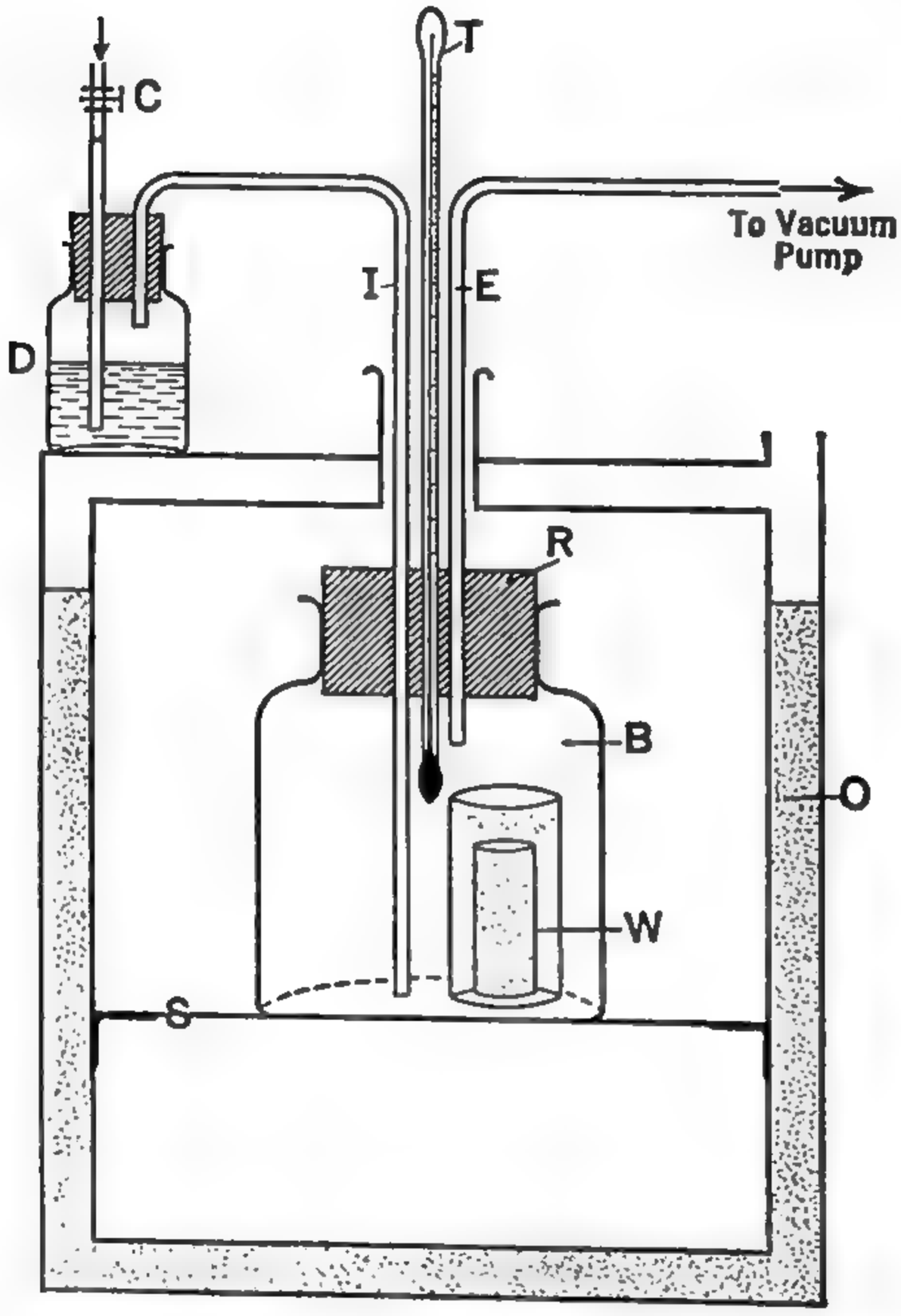


FIG. 17. Browne's method of vacuum drying.

Browne's Method of Vacuum Drying. When one of the specially constructed types of vacuum drying oven is not available, Browne has found the following arrangement (Fig. 17), which is easily constructed from ordinary laboratory materials, to be perfectly efficient.

The vacuum chamber consists of a large-mouth bottle *B* of heavy glass, which is supported by the shelf *S* of an ordinary water oven *O*. The mouth of the bottle is closed by a tight-fitting rubber stopper *R* whose three holes permit the insertion, through the top opening of the oven, of the tubes *I* and *E* and the thermometer *T*. The bottle is easily fitted, and detached from the stopper by first withdrawing the shelf, the shelf being shoved into position again when the bottle is in place. The current of air entering by tube *I* to the bottom of the vacuum bottle is controlled by a clamp pinchcock *C* and freed of moisture by a gas drier *D*. The exit air from the vacuum bottle passes by the tube *E* to the vacuum pump or aspirator.

For absorbing the sugar-containing liquid, asbestos in perforated brass or copper tubes is used. The tubes measure 9 cm. long by 2 cm. in diameter, and are nearly filled with freshly ignited asbestos tightly packed with a rod against the sides in the upper half of the tube so as to leave a central cavity.

Each tube thus prepared is placed in a glass-stoppered weighing bottle of sufficient size, and the whole weighed. About 5 ml. of the liquid to be analyzed is then delivered from a pipette into the cavity in the asbestos, the object of the cavity being to secure a rapid absorption and even distribution of the liquid through the asbestos. The weighing bottle is then immediately stoppered and reweighed, the increase in weight being the amount of substance taken. After the stopper is removed the weighing bottle with tube is placed in the vacuum bottle, as shown by *W* in the diagram, and the temperature raised to 70°C. During the first few hours of drying a brisk current of air is drawn through the vacuum bottle in order to remove the large excess of moisture first given off. In the last stages of the drying the air current is decreased and the vacuum kept at about 25 in. At the end of a few hours the weighing bottle is removed, allowed to cool in a desiccator, and then restoppered and weighed. The bottle is then redried for a second short period to determine if all moisture has been expelled.

Vacuum Drying Method of the Association of Official Agricultural Chemists. The directions for drying materials which contain fructose, on pumice stone or sand, are the same as those for drying at the temperature of boiling water (p. 28), except that a temperature of 70° C. and a pressure not exceeding 50 mm. of mercury are specified, and that trial weighings at 2-hour intervals are prescribed for drying on sand.

Cane products, when dried at 70° C. in vacuo, often are found to undergo a continuous loss of weight. In such cases the temperature of drying should be reduced still further. De Whalley[12] has proposed to use 60° C. at a pressure of not over 50 mm. of mercury. To hasten the removal of the water vapor a slow current of dry air is passed through the oven.

Rice[13] recommends drying at room temperature over sulfuric acid in a vacuum desiccator at about 25 mm. pressure. Under these conditions constant weight was obtained in about 48 hours, and no further loss was observed even after 10 days. De Whalley has found that glucose loses its water of crystallization at 60° C., but not at room temperature. In the latter case it is therefore necessary to deduct from the dry substance found the water of crystallization in the glucose present. But it is possible that some of the salts in the product may or may not lose their water of crystallization at either temperature, and this introduces a new source of error.

[12] "Proceedings Ninth Session, International Commission for Uniform Methods of Sugar Analysis," *Intern. Sugar J.*, 39, 36s (1937).

[13] *Ind. Eng. Chem., Anal. Ed.*, 1, 31 (1929).

Drying Method of Rice and Boleracki.[14] These authors found that cane molasses, sirups, or honeys can be dried at 70° C. in vacuo to constant weight in 2 to 4 hours, if placed between two metal plates and rolled out to a thin film. The results agreed closely with those obtained by the official method of the Association of Official Agricultural Chemists, by drying on sand in vacuo at 70° C. The procedure is described as follows:

Two silver plates, 10 by 15 cm., and 0.0007 cm. in thickness, are cut with rounding corners and provided with a light skeleton frame of aluminum. One plate is laid on the table and a drop of sample of about 0.25 to 0.3 g. placed about 5 cm. from one end. The plates are then placed together with the sample between and rolled toward the far end a few times to make them stick together. They are then placed in the frame and weighed to obtain the weight of sample taken. They are taken out of the frame and placed upon a piece of warm (60° to 70° C.) plate glass and rolled with a rubber-faced roller, such as is used to mount photographs, to maximum distribution, and then finished off with a wooden roll whereby considerably more spreading can be attained. With a little practice a proper spread should not require over 2 to 3 minutes. It is possible to feel the material push ahead of the wooden roll, and the proper spread can be accomplished without separating the plates. It is advisable to separate them partly to check the spread until one becomes accustomed to the method. The rubber roll should be longer than the plates are wide, and the wooden roll 2.5 to 5 cm. in length. The wooden roll must be used only after the rubber roll or the plates will begin to dish. Results indicate that if constant weight is not obtained in 2 to 4 hours, the rolling was not sufficient. When no further spreading is possible, the plates are pulled apart and placed in the drying oven. When it is necessary to weigh, they must be placed together as quickly as possible with the sample side in, slipped into the frame and cooled in a desiccator. Cooling as well as heating is very quick.[15]

Drying of Sugars in Vacuo. In 1900 the International Commission for Uniform Methods of Sugar Analysis adopted,[16] for moisture determinations in normal beet sugars, the method of drying at 105° to 110° C. under atmospheric pressure. But at the Ninth Session in 1936,[17] it decided to recommend vacuum drying as the standard procedure for raw as well as refined sugars from either cane or beet.

Ten grams of the sugar is weighed into a metal dish with tight-fitting cover, 2 inches wide by 1 inch high. The sample is dried for 5 hours in a vacuum oven at 60° C. and a pressure of not more than

[14] *Ind. Eng. Chem., Anal. Ed.*, 5, 11 (1933).

[15] The entire apparatus is manufactured by Eimer & Amend, New York, N. Y.

[16] "Proceedings of the Paris Session," 1900.

[17] "Proceedings of the Ninth Session," *Intern. Sugar J.*, 39, 36s (1937).

50 mm. of mercury. The oven is to be bled with a current of dry air to facilitate the removal of the water vapor. The drying is then continued for 1-hour intervals until the loss in weight is less than 1 mg. If other drying methods are preferred the conditions must be adjusted so as to give the same results as the standard method. The Association of Official Agricultural Chemists has adopted the vacuum method described, with slight modifications.[18] The weight of sample is reduced to 2 to 5 g.; the initial drying period is shortened to 2 hours, and the weight is considered constant when the loss after an additional drying period of 1 hour does not exceed 2 mg.

Method of Brown, Sharp, and Nees for Beet House Sirups.[19] Reduced pressure may also be used to hasten the drying of materials that can safely be heated to temperatures above 70° C. This principle is used in the method of Brown, Sharp, and Nees for determining moisture in beet house sirups with a precision of 0.1 per cent. The method is as follows:

> Digest clean sea sand that will pass a 40-mesh sieve but not a 60-mesh screen in strong hydrochloric acid, wash free from acid, dry and ignite. Place 25 to 30 g. of the sand in an aluminum dish, provided with a short stirring rod and cover. A dish 50 mm. in diameter and 35 mm. high is very satisfactory. Stir into the sand about 0.5 g. of powdered graphite, free from oil. Dry the dish in an oven overnight, cool in a desiccator, and weigh. Weigh into the dish, from a weighing burette, an amount of sirup containing 0.5 to 0.7 g. of dry substance. Mix the contents of the dish thoroughly and place in a vacuum oven which permits no appreciable air leak. Dry at a temperature of 90° and at a pressure of 125 mm. or less in an atmosphere of dried carbon dioxide, feeding about 3 to 4 cubic feet (at atmospheric pressure) of carbon dioxide per hour to the oven. Heat the samples for 72 hours or more; remove from the oven, transferring the dishes quickly to individual desiccators containing fresh phosphorus pentoxide, allow to stand in the desiccator 3 days or more, and weigh rapidly after removing from the desiccator.

Sea sand is used because it has no capillary spaces from which moisture is difficult to remove. The graphite is added to eliminate charges of static electricity accumulating on the sand and affecting its weight. The drying is carried out in an atmosphere of carbon dioxide to prevent oxidation of organic matter which would increase the weight. The sample is kept in a desiccator for at least 3 days before weighing, because constant weight is not obtained in a shorter time. Individual desiccators are necessary because, if several samples are placed in the same desiccator, all the samples weighed after the

[18] *J. Assoc. Official Agr. Chem.*, **21**, 89 (1938).
[19] *Ind. Eng. Chem.*, **20**, 945 (1928).

first one pick up moisture when the desiccator is opened. This method gives reliable results to within 0.06 per cent with beet house sirups low in raffinose; it is not directly applicable to sirups high in raffinose or to cane products.

Determination of Moisture in Sugar Materials Which Contain Water of Hydration

Difficulty is sometimes experienced in dehydrating sugars such as glucose, lactose, maltose, and raffinose, which crystallize with one or more molecules of water of crystallization. The principal precaution to be observed in drying such sugars is not to raise the temperature in the first stages of the process above the melting point of the hydrate, otherwise the sugar will liquefy to a thick viscous mass from which it is difficult to expel the last traces of water without decomposition.

For drying glucose hydrate, $C_6H_{12}O_6 + H_2O$, the sugar is spread in a thin layer and gently warmed at 50° to 60° C. for several hours, when most of the water will be removed without melting of the crystals. The sugar is then gradually heated to about 105° C., when the last traces of water will be expelled, with no evidence of liquefaction.

For drying raffinose hydrate, $C_{18}H_{32}O_{16} + 5\ H_2O$, the finely powdered sugar is first warmed to 80° C. for several hours and then the temperature gradually raised to about 105° C. The preliminary drying may be hastened greatly by heating the sugar in a vacuum oven.

Maltose hydrate, $C_{12}H_{22}O_{11} + H_2O$, gives off its water very incompletely at 100° C. under atmospheric pressure, and vacuum dehydration is necessary. The sugar is gently heated under a strong vacuum at 90° to 95° C., and then after a few hours the temperature is raised to between 100° and 105° C.

Lactose hydrate, $C_{12}H_{22}O_{11} + H_2O$, retains its water of crystallization unchanged at 100° C. under atmospheric pressure. It is therefore customary in analytical work to estimate lactose as the hydrate. Lactose may be dehydrated, however, by gently heating the finely pulverized sugar in a strong vacuum to a temperature of 125° to 130° C.

The method of drying devised by Lobry de Bruyn and van Leent,[20] and used by Brown, Morris, and Millar,[21] and also by Walker,[22] is to weigh the finely powdered sugar in a small flask and connect the flask by a T tube to a bottle containing phosphorus pentoxide, P_2O_5, as a dehydrating agent. The open branch of the T tube is connected with

[20] *Rec. trav. chim.*, 13, 218 (1894).
[21] *J. Chem. Soc. Trans.*, 71, 76 (1897).
[22] *J. Am. Chem. Soc.*, 29, 541 (1907).

a strong vacuum; the flask containing the sugar is then placed in an oil bath and the temperature gently raised to the point desired. Walker found that lactose under these conditions, after heating 1 hour at 80° C. and then 1 hour at 130° C., remained perfectly white, but upon heating to 140° C. the sugar became tinged with brown, showing signs of decomposition.

The method of Lobry de Bruyn and van Leent has also been successfully employed by Rolfe and Faxon[23] for determining the total carbohydrates in acid-hydrolyzed starch products. In the modified apparatus of Rolfe and Faxon the T tube is provided with a three-way stopcock, which allows the great excess of water first given off to be removed without coming in contact with the phosphorus pentoxide.

Precautions in Handling Anhydrous Sugar Products. Many carbohydrate and sugar-containing materials, because of their great hygroscopic action in an anhydrous condition, attract atmospheric moisture with great avidity, and such products must be removed to the desiccator with the utmost quickness as soon as the drying oven is opened. Desiccators for retaining sugar products in an anhydrous condition must be recharged frequently with sufficient quantities of concentrated sulfuric acid or other effective dehydrating agent. The weighing bottle, or other receptacle which contains the sugar products, must also be tightly closed during weighing in order to prevent absorption of moisture from the air.

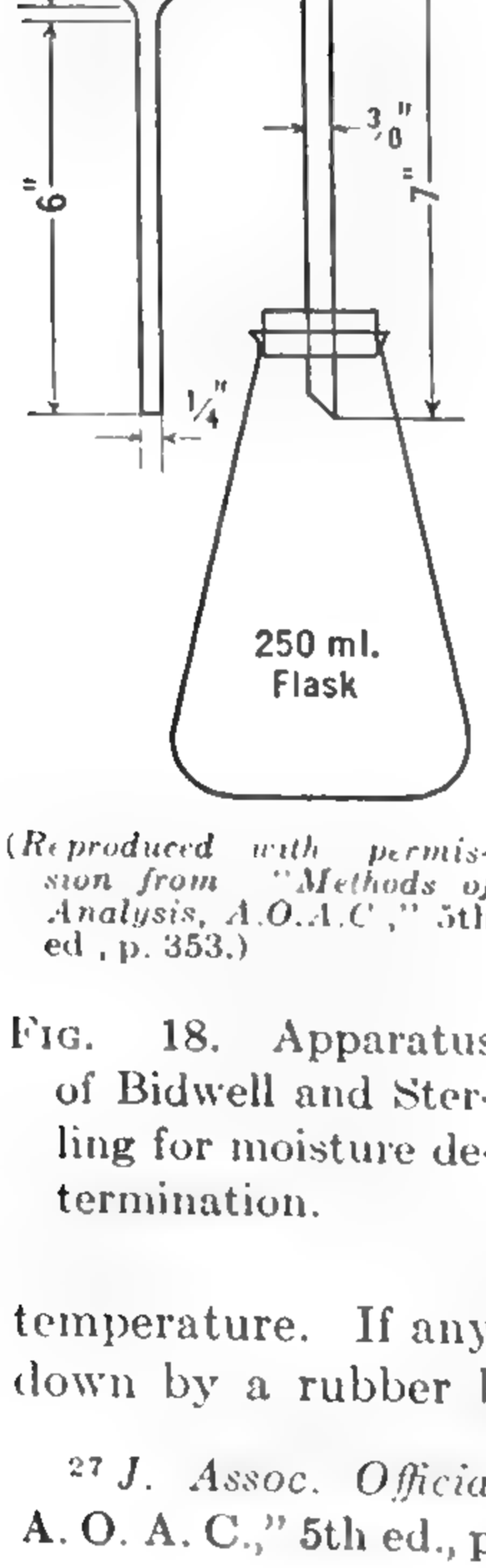
DIRECT METHOD FOR DETERMINING WATER IN SUGAR PRODUCTS

Because of the uncertainty which sometimes attends an indirect method of moisture determination, several methods have been proposed for the distillation and direct measurement of the water in sugar-containing substances. The distillation method of removing and measuring water was first applied by Senger[24] in 1902 to the analysis of tar. Its application to sugar products was first made in 1904 by Testoni,[25] who determined the water in molasses by measuring the quantity that was obtained upon distilling the product with turpentine of b.p. 160°. Later, in 1917, Van der Linden, Kauffman, and Leistra[26] devised a direct method for determining the water in molasses and other sugar-factory products by distilling 50 g. of the product with 350 ml. of xylene in a copper distillation flask connected to an upright condenser,

[23] *J. Am. Chem. Soc.*, 19, 698 (1897).
[24] *J. Gasbeleucht.*, 45, 841 (1902).
[25] *Staz. sper. agrar. ital.*, 37, 366 (1904).
[26] *Arch. Suikerind.*, 25, 951 (1917).

the lower end of which discharged into a 250-ml. measuring cylinder graduated to 0.05 ml. The distillation was so regulated that the first 100 ml. of distillate passed over in 45 minutes and the second 100 ml. in 15 minutes. The distillation was then stopped and the volume of water beneath the supernatant xylene measured. Slight empirical corrections for distortion of meniscus and loss of water by volatilization and absorption had to be made for the volume of water in the receiver.

On account of the liability of certain sugars, more especially fructose, to decompose at the boiling point of xylene (b.p. 139°), Bidwell and Sterling[27] have modified the distillation method by employing toluene (b.p. 110.5°) as the immiscible liquid with which the product is boiled. The diagram and dimensions of the Bidwell-Sterling apparatus are shown in Fig. 18; the directions for making the analysis are as follows:



(*Reproduced with permission from "Methods of Analysis, A.O.A.C.," 5th ed., p. 353.*)

FIG. 18. Apparatus of Bidwell and Sterling for moisture determination.

Introduce into a 250-ml. Pyrex Erlenmeyer flask sufficient sample to give from 2 to 5 ml. of water. If the sample is likely to bump, add enough dry sand to cover the bottom of the flask. Add sufficient toluene to cover the sample completely, usually about 75 ml., and connect the apparatus as shown in Fig. 18. Fill the receiving tube with toluene by pouring through the top of the condenser. Bring to a boil and distil slowly, about 2 drops per second, until most of the water has passed over; then increase the rate of distillation to about 4 drops per second. When all the water is apparently over, wash down the condenser by pouring toluene in at the top, continuing the distillation a short time to ascertain whether any more water will distil over; if it does, repeat the washing-down process. If any water remains in the condenser, remove it by brushing down with a tube brush attached to a copper wire and saturated with toluene, washing down the condenser at the same time. The entire process is usually completed within an hour. Allow the receiving tube to come to room temperature. If any drops adhere to the sides of the tube they can be forced down by a rubber band wrapped around a copper wire. Read the volume

[27] *J. Assoc. Official Agr. Chem.*, **8**, 295 (1924/25); "Methods of Analysis, A. O. A. C.," 5th ed., p. 353, 1940.

of water and calculate to percentage. The tube is calibrated in tenths of a milliliter, and the column can be read to hundredths with reasonable accuracy.

It is necessary to have the condenser and receiving tube chemically clean in order to prevent an undue quantity of water sticking to the condenser and drops of water adhering to the sides of the receiving tube. Clean with chromic-sulfuric acid, rinse with water, then with alcohol, and dry in an oven.

The correction for loss of water by volatilization and absorption is found by making a blank distillation of 75 ml. toluene with an accurately measured amount of water. The correction in the experiments of Bidwell and Sterling was +0.02 ml.

A comparison of water determinations upon various sugar-containing products by the toluene distillation method and vacuum-oven method is given in Table X, which is taken from the results by Bidwell and Sterling.

TABLE X

WATER DETERMINATION IN SUGAR PRODUCTS BY TOLUENE DISTILLATION AND BY VACUUM OVEN

Product	Per Cent Water	
	Toluene Distillation Method	Vacuum Oven Method
Raisins	13.10	13.09
Raisins	15.00	13.17
Raisins	15.40	13.55
Raisins	13.30	11.06
Beet molasses	21.90	21.92
Cane molasses	20.42	20.34
Honey	19.59	19.40
Dried apples	13.99	12.34

The considerably higher results obtained by the distillation method upon some of the samples are probably due to the splitting off of water of decomposition from fructose at the temperature of the boiling toluene, although with liquid fructose-containing products, such as cane molasses and honey, a much better agreement was found.

Other investigators have obtained with this method much higher results for moisture in cane blackstraps and refiner's sirups than by drying on sand in vacuo at 70° C. Rice[28] observed that the blackening and decomposition which usually occur in such cases can be reduced by adding 10 g. of dry sodium oxalate to the mixture in the flask. Still better results were obtained with dried Filter-Cel. Five grams of this material is first placed in the flask, the molasses or sirup

[28] *Ind. Eng. Chem., Anal. Ed.*, 1, 31 (1929).

is weighed in, and then another 5 g. of Filter-Cel is added. The analysis is carried out as prescribed by Bidwell and Sterling, the flask being heated in an oil bath at 127° C. Most of the water distils over in 1 hour, and at the end of 3 hours the quantity of distillate agrees closely with the loss in weight by drying in vacuo at 70° C., the maximum deviation amounting to 0.3 per cent for refiner's sirup containing about 25 per cent of invert sugar.

Rice's modification has been adapted for use on corn products by Fetzer, Evans, and Longenecker.[29] The procedure is as follows:

> Approximately 10 g. of dry Filter-Cel is placed in the dry 250-ml. Erlenmeyer flask, and the weight is obtained. Then approximately 20 to 25 g. of corn sirup is run into the flask on the Filter-Cel and the flask is reweighed to find the exact weight of sirup taken. Approximately 10 g. more of Filter-Cel is added; a small dry test tube (1.27 by 10.2 cm.) is introduced by means of a pencil, the eraser end being in the test tube. Using the test tube pencil as a stirring rod, the corn sirup and the Filter-Cel are mixed together. The pencil is then removed, leaving the test tube in the flask. Approximately 75 ml. of toluene is added, the flask is connected to the receiver and condenser, the receiver is filled with toluene, and the distillation is started. An oil or glycerol bath is used, maintaining a temperature of about 127° C.

The determination is completed in about 6 hours, and the results are claimed to be the most reliable of all methods for moisture in corn products.

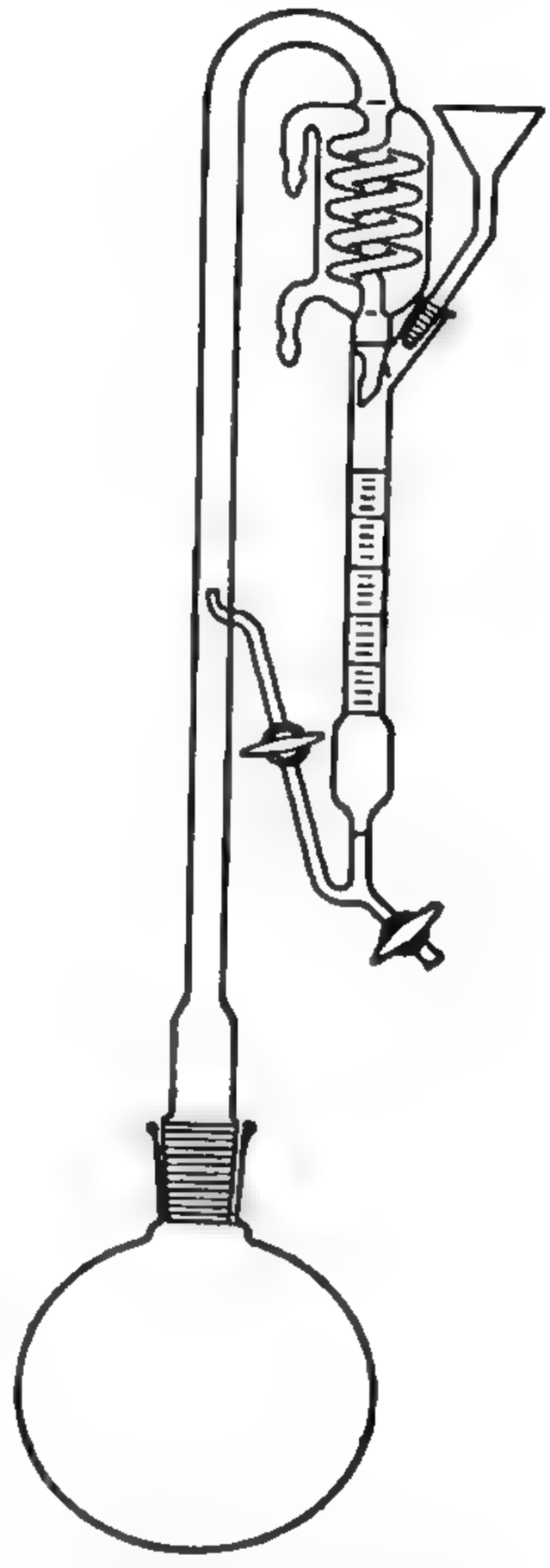
Method of Thielepape and Fulde for Beet Products. Objections have been raised against the use of xylene and toluene because of the fire hazard. Another objection is that they are lighter than sirups and molasses. As a consequence these products are in direct contact with the bottom of the flask, causing overheating. For this reason Thielepape and Fulde[30] introduced the use of alkyl and alkylene chlorides. Good results were obtained in moisture determinations on beet products with a mixture of 1 volume of trichloroethylene with 2 volumes of tetrachloroethane, which boils at 112° to 115° C. and has a specific gravity of 1.55. But this mixture was later abandoned, because of the high toxicity of tetrachloroethane; tetrachloroethylene, which is only very slightly toxic, was substituted for the mixture.[31] This compound has a specific gravity of 1.62 and boils at 119° C.

The apparatus of Lundin,[32] Fig. 19, is used for the distillation. The receiver consists of a bulb with two marks, of 20-ml. capacity,

[29] *Ind. Eng. Chem., Anal. Ed.*, **5**, 81 (1933).
[30] *Z. Ver. deut. Zucker-Ind.*, **81**, 567 (1931).
[31] *Z. Ver. deut. Zucker-Ind.*, **82**, 665 (1932); **87**, 333 (1937).
[32] *Chem. Ztg.*, **55**, 762 (1931).

and a measuring tube holding 10 ml. more, graduated in 1/20 ml. The tetrachloroethylene runs back automatically through a connecting tube with stopcock. All ground-glass joints should be greased with a thin film of stopcock grease, and it is advisable to insulate the upper part of the vapor tube, between the condenser and the inlet of the overflow tube, with asbestos.

About 150 to 200 ml. of tetrachloroethylene is placed in the 500-ml. flask, the weighed sample is added, and the apparatus put together. Tetrachloroethylene is also poured into the measuring tube, with the stopcock in the overflow tube open, until the liquid runs over into the flask. The cooling water is turned on and the distillation started. When no more water passes over, the drop of tetrachloroethylene that usually collects on top of the water is removed by slightly tapping the measuring tube, or by pushing the drop down with a thin wire having a loop at the end of it and introduced through the filling funnel. The amount of the water must be either less than 10 ml. or between 20 and 30 ml. In the former case its exact quantity is determined by the difference in level between the surface of the tetrachloroethylene and the water, or else by adjusting the lower water level to the first mark above the bulb. If there is more than 20 ml. of water, the reading is taken from the lower mark, below the bulb. When the determination is finished, the water is run out through the lower stopcock, and the flask is cleaned and made ready for the next determination. The apparatus must be calibrated before use by distilling known amounts of water.



(Reproduced with permission from Z. Ver. deut. Zucker-Ind., 87, 338.)

FIG. 19. Lundin's apparatus for moisture determination.

The tetrachloroethylene can be used several times. When it becomes discolored it is purified by a simple distillation. The apparatus should be cleaned from time to time with chromic acid mixture.

Beet molasses or fillmass requires from 1 to 2 hours for the distillation; evaporator sirup, 45 to 60 minutes; raw sugars, from 30 to 90 minutes; cossettes, from 30 to 45 minutes; press cake, 30 to 50 minutes. The results checked well with those obtained by the usual drying method for beet products, the maximum difference for molasses being 0.36 per cent.

If the boiling point of tetrachloroethylene should be found too high for readily decomposed substances, it may be lowered by the addition of trichloroethylene.

Among other, indirect methods which have been tried for the estimation of water, especially in dried beet pulp, may be mentioned one in which the pulp is heated with calcium carbide and the evolved acetylene measured, and another in which the pulp is digested with absolute alcohol and the specific gravity of the alcohol-water mixture determined. Neither of these methods has given satisfactory results.[33]

[33] Spengler, Matthies, and Tödt, *Z. Ver. deut. Zucker-Ind.*, **84**, 941 (1934).

CHAPTER III

DENSIMETRIC METHODS OF ANALYSIS

The quantity of matter in a unit volume of substance is called the absolute density of that substance. If m is the mass and V the volume of a given substance, its absolute density D will be $D = m/V$. The ratio between the masses of equal volumes of a substance and of some standard material is the relative density of that substance. Since, however, the masses of two bodies at any one place are proportional to their weights, the relative density S of a given substance may be expressed as $S = w/W$, where w and W are the weights respectively of equal volumes of the substance and standard material. Relative density is commonly known as specific gravity, and, since the standard substance of comparison is nearly always water, specific gravity is commonly defined as a number indicating how much heavier a substance or solution is than an equal volume of water.

The determination of specific gravity is one of greatest importance in the analysis of sugars; its value consists in the fact that solutions of different sugars of equal concentration have about the same density. The following densities (less accurately specific gravities) relate to 10 per cent solutions of nine different sugars at 20° C. with reference to water at 4° C.: arabinose 1.0379, glucose 1.0377, fructose 1.0385, galactose 1.0379, sorbose 1.0381, sucrose 1.0381, maltose 1.0386, lactose 1.0376, raffinose 1.0375. It will be noted that the specific gravity of each sugar solution is but little removed from the average 1.0380, which is almost the same as that of sucrose. It is possible, therefore, by means of specific-gravity tables established for solutions of pure sucrose to determine very closely the percentage of dissolved substance for any sugar or mixture of sugars in aqueous solution.

Units of Volume. The unit of volume employed in sugar analysis is the cubic centimeter (cc.). This unit is differently defined, and the chemist must distinguish carefully between (1) the original cubic centimeter; (2) the metric or true cubic centimeter, preferably called milliliter (ml.); (3) the Mohr cubic centimeter; and (4) the reputed cubic centimeter.

The original cubic centimeter is the volume of a cube the edge of which is 1 cm. long.

The milliliter, generally termed *metric or true cubic centimeter* in the sugar literature, is defined as the volume occupied by 1 g. of pure water at 4° C., the temperature of maximum density ($D = 1.000000$). The water is under a pressure of 760 mm. of mercury, and the weighings are reduced to vacuo. One milliliter equals 1.000027 original cubic centimeter. The difference between the two is due to the fact that, although the kilogram was first defined as the mass of 1 cubic decimeter of water, it is now defined as the mass of the prototype kept at the International Bureau of Weights and Measures in Sèvres. At 20° C. the metric or true cubic centimeter is equivalent to the volume occupied by 0.998234 g. of water weighed in vacuo, or 0.997176 g. of water weighed in air with brass weights. The term milliliter is employed in the present work.

The Mohr cubic centimeter is defined as the volume occupied by 1 g. of water weighed in air with brass weights at 17.5° C. One Mohr cubic centimeter, as thus defined, is equivalent to 1.00234 ml.

The reputed cubic centimeter, a term introduced by Brown, Morris, and Millar,[1] is defined as the volume at 15.5° C. of 1 g. of water weighed in air with brass weights. One reputed cubic centimeter, as thus defined, is equivalent to 1.00198 ml.

The true or metric cubic centimeter was adopted as the standard unit of volume by the International Commission for Uniform Methods of Sugar Analysis at its meeting in Paris, 1900.

Specific-Gravity Tables for Sugar Solutions

Various tables have been established by different observers which give the specific gravity (sp. gr.) of cane-sugar solutions for different concentrations. These tables are expressed in several ways; they vary according to the temperature which is selected for the determination, 15° C., 17.5° C., or 20° C. usually being taken, and also as to whether the weight of water at 4° C. is used for comparison, in which case the values are numerically equal to the density, or water at 15° C., 17.5° C., and 20° C., when the use of the term specific gravity solely is permissible, but not the term density. In expressing specific gravity it is customary to indicate the system employed by writing the temperature of the solution above that of the water; thus $^{15°}_{4°}$, $^{20°}_{4°}$, $^{17.5°}_{17.5°}$, $^{20°}_{20°}$, etc. A further distinction must be made between values based on weights in air and values derived from weights in vacuo. There are thus four different values for the relationships of the weight of a given

[1] *J. Chem. Soc.*, 71, 78 (1897).

volume of water (vw) to the weight of an equal volume of sugar solution (vs) at any temperature t.

$$\text{True density} = \frac{vs^t}{vw^{4^\circ}} \text{ (weights in vacuo)}$$

$$\text{Apparent density} = \frac{vs^t}{vw^{4^\circ}} \text{ (weights in air)}$$

$$\text{True specific gravity} = \frac{vs^t}{vw^t} \text{ (weights in vacuo)}$$

$$\text{Apparent specific gravity} = \frac{vs^t}{vw^t} \text{ (weights in air)}$$

These values are mutually convertible. Since the true density at 20° C., for instance, is the weight in vacuo of 1 ml., and the apparent density the weight in air of 1 ml., the first is converted into the second by correcting for the buoyancy. If M is the weight in vacuo, and W the apparent weight in air, then

$$M = W\left[1 + \frac{\rho}{d_2}\left(\frac{d_2 - d_1}{d_1 - \rho}\right)\right]$$

where ρ is the density of the air (0.0012046 at 20° C. and 760 mm. pressure), d_1 the density of the solution, and d_2 the density of the brass weights (8.4). The true specific gravity is similarly converted into the apparent specific gravity by the same formula.

The true density $\frac{20^\circ}{4^\circ}$ is converted into the true specific gravity $\frac{20^\circ}{20^\circ}$ by dividing by the true density of water at 20°, 0.998234, and the apparent density $\frac{20^\circ}{4^\circ}$ into the apparent specific gravity $\frac{20^\circ}{20^\circ}$ by dividing by the apparent density of water at 20° C., 0.997176.

The term specific gravity, without further qualification, usually denotes apparent specific gravity.

In Table XI the specific gravities of sucrose solutions at several concentrations are given according to the calculations of different authorities.

Various formulas have been worked out for expressing the relationship between the specific gravity and percentage by weight of dissolved sucrose. Gerlach for specific gravity $\frac{17.5^\circ}{17.5^\circ}$ has expressed the relationship by the equation

$$y = 1 + 0.00386571327\,x + 0.00001414091906\,x^2 + 0.000000003287946571 76\,x^3$$

in which y is the specific gravity and x the percentage of sugar.

Scheibler has recalculated Gerlach's equation for sugar solutions of different temperatures with the following results:

Temperature	
0°	$y = 1 + 0.003976844\,x + 0.0000142764\,x^2 + 0.000000029120\,x^3$
10	$y = 1 + 0.003915138\,x + 0.0000139524\,x^2 + 0.000000032728\,x^3$
15	$y = 1 + 0.003884496\,x + 0.0000139399\,x^2 + 0.000000033806\,x^3$
20	$y = 1 + 0.003844136\,x + 0.0000144092\,x^2 + 0.000000030912\,x^3$
30	$y = 1 + 0.003796428\,x + 0.0000145456\,x^2 + 0.000000030664\,x^3$
40	$y = 1 + 0.003764028\,x + 0.0000143700\,x^2 + 0.000000035192\,x^3$
50	$y = 1 + 0.003722992\,x + 0.0000148088\,x^2 + 0.000000032440\,x^3$
60	$y = 1 + 0.003683112\,x + 0.0000155904\,x^2 + 0.000000026368\,x^3$

TABLE XI

SPECIFIC GRAVITY OF SUCROSE SOLUTIONS BY DIFFERENT AUTHORITIES

Sucrose, per cent by weight	Balling-Brix, $d\,{}^{17.5^\circ}_{17.5^\circ}$ C.	Gerlach, $d\,{}^{17.5^\circ}_{17.5^\circ}$ C.	Gerlach-Scheibler, $d\,{}^{15^\circ}_{15^\circ}$ C.	German Imperial Commission	
				$d\,{}^{15^\circ}_{15^\circ}$ C.	$d\,{}^{20^\circ}_{4^\circ}$ C.
0	1.00000	1.00000	1.00000	1.00000	0.99823
5	1.01970	1.01969	1.01978	1.01973	1.01785
10	1.04014	1.04010	1.04027	1.04016	1.03814
15	1.06133	1.06128	1.06152	1.06134	1.05917
20	1.08329	1.08323	1.08354	1.08328	1.08096
25	1.10607	1.10600	1.10635	1.10604	1.10356
30	1.12967	1.12959	1.12999	1.12962	1.12698
35	1.15411	1.15403	1.15448	1.15407	1.15128
40	1.17943	1.17936	1.17985	1.17940	1.17645
45	1.20565	1.20559	1.20611	1.20565	1.20254
50	1.23278	1.23275	1.23330	1.23281	1.22957
55	1.26086	1.26086	1.26144	1.26091	1.25754
60	1.28989	1.28995	1.29056	1.28997	1.28646
65	1.31989	1.32005	1.32067	1.31997	1.31633
70	1.35088	1.35117	1.35182	1.35094	1.34717
75	1.38287	1.38334	1.38401	1.38286	1.37897

One of the best-known tables for the specific gravity of sugar solutions is that of Balling[2] ($^{17.5^\circ}_{17.5^\circ}$), published in 1854, which served as a basis for the better-known and more complete table of Brix, whose name is now almost universally given to the percentages of sugar or dissolved solids (degrees Brix) derived by densimetric means. Another well-known table is that of Gerlach[3] ($^{17.5^\circ}_{17.5^\circ}$), published in 1863–64, which served as a basis for Scheibler's[4] table calculated to $^{15^\circ}_{15^\circ}$. The most recent and most accurately established tables are those of the German Imperial Commission[5] upon Standards, based upon the deter-

[2] *Z. Ver. deut. Zucker-Ind.*, **4**, 304 (1854).
[3] *Dingler's Polytech. J.*, **172**, 31 (1864).
[4] *Neue Zeitschrift*, **25**, 37, 185 (1890).
[5] *Z. angew. Chem.*, 1898, 774; *Z. Ver. deut. Zucker-Ind.*, **50**, 982 to 1079 (1900).

minations of Plato and published in 1898 and 1900. These tables give the percentages of sucrose for true specific gravities at $\frac{15°}{15°}$ and $\frac{r°}{15°}$, and for true densities at $\frac{20°}{4°}$. The $\frac{20°}{4°}$ table, which was established according to the requirements of the Fourth International Congress of Applied Chemistry (Paris, 1900), is given in the Appendix (Table 1).[6]

Domke[7] has published a table, based on the same fundamental data and giving the apparent specific gravity, at $\frac{20°}{20°}$, for solutions containing from 0 to 90 per cent sucrose. (See Appendix, Table 3; recalculated to fifth decimal place and extended to 95 per cent sucrose.) A similar table, showing true densities at $\frac{20°}{4°}$ and true specific gravities at $\frac{20°}{20°}$, extended to 100 per cent sucrose, is to be found in Technological Paper 115 of the U. S. Bureau of Standards.

The tables of the German Imperial Commission have since been enlarged by Sidersky[8] so as to give the grams of sugar for 100 g. and also for 100 ml., of solution for $\frac{t°}{4°}$ and $\frac{t°}{15°}$ between 10° and 30° C. and for concentrations between 0° and 30° Brix.

For convenient use in the tropics, tables valid at higher temperatures have been calculated. One of these, by Douwes-Dekker and Erlee,[9] gives the true density, $\frac{27.5°}{4°}$; another by Sidersky[10] the true density, $\frac{27°}{4°}$; and a third, for Hawaii,[11] apparent specific gravity, $\frac{27.5°}{27.5°}$.

Influence of Temperature upon the Specific Gravity of Sugar Solutions. With increase of temperature, sugar solutions expand in volume and the specific gravity becomes correspondingly less. The coefficient of cubical expansion of sugar solutions varies according to concentration. Josse and Remy[12] give the coefficients shown in Table XII for different sugar solutions between 15° and 25° C.

The mean coefficient of expansion (γ) of a solution containing p per cent of sucrose for temperatures between 10° and 27° C. can be found by Schönrock's[13] formula with a probable error of only ± 0.000006.

$$\gamma = 0.000291 + 0.0000037\ (p - 23.7) + 0.0000066\ (t - 20) - 0.00000019\ (p - 23.7)\ (t - 20)$$

The value of γ being known, the specific gravity dt at temperature t

[6] See also "International Critical Tables," Vol. II, p. 343, 1927.

[7] *Z. Ver. deut. Zucker-Ind.*, 62, 302 (1912).

[8] "Les densités des solutions sucrées à différentes températures," Paris, 1908.

[9] *Arch. Suikerind.*, 38, III, 697 (1930).

[10] *Bull. assoc. chim.*, 52, 432 (1935).

[11] "Methods of Chemical Control of the Association of Hawaiian Sugar Technologists," 2nd ed., p. 94, 1931.

[12] *Bull. assoc. chim. sucr. dist.*, 19, 302 (1901/02).

[13] *Z. Ver. deut. Zucker-Ind.*, 50, 419 (1900).

TABLE XII

COEFFICIENTS OF CUBICAL EXPANSION FOR SUGAR SOLUTIONS

d 15° C.	*d* 25° C.	Concentration	Coefficient
1.02425	1.02211	6.32	0.0002052
1.05100	1.04365	12.75	0.0002100
1.10025	1.09744	23.88	0.0002250
1.14782	1.14452	33.71	0.0002574
1.19875	1.19500	43.81	0.0002896
1.25110	1.24718	53.37	0.0003153
1.30384	1.29962	62.39	0.0003262
1.33025	1.32591	66.74	0.0003289

can be calculated from the specific gravity dt_0 at temperature t_0 by the equation

$$dt = dt_0 + [dt_0 \times \gamma(t_0 - t)]$$

In the employment of temperature corrections in densimetric methods of analysis, it is more customary to apply the correction to the percentage of sugar (degrees Brix) than to the specific gravity. The correction is to be added if the temperature is above, and to be subtracted if the temperature is below, the standard degree of the table (17.5° C. for the old Brix tables and 20° C. for the new tables of the German Commission). Lists of such corrections are affixed to the standard tables of specific gravities.[14]

Densities of Glucose Solutions. Jackson[15] gives the following formula for the true density, at 20° C., of glucose solutions containing p per cent sugar by weight (in vacuo), valid for the range from $p = 5$ to $p = 30$:

$$d_4^{20} = 0.99840 + 0.003788\,p + 0.00001412\,p^2$$

From this formula the following table has been calculated:

p	5	10	15	20	25	30
d_4^{20}	1.01769	1.03769	1.05840	1.07981	1.10193	1.12475

These values are lower throughout than those for sucrose solutions of the same concentration.

Similar results, obtained by Riiber,[16] but based on c, grams glucose (in vacuo) in 100 ml. solution, are shown in Table XIII. The values of p, corresponding to c, are also given, and for comparison the equivalent density figures from Jackson's equation.

[14] Appendix, Table 2, for 20° C.

[15] *Bur. Standards Sci. Paper* **293**, 1916.

[16] *Ber.*, **56B**, 2185 (1923).

TABLE XIII

c	d_4^{20} Riiber	p	d_4^{20} Jackson
5	1.017395	4.9145	1.01736
10	1.036443	9.6484	1.03626
15	1.055371	14.2458	1.05509
20	1.074178	18.6189	1.07382
25	1.092861	22.8757	1.09244

Riiber's values exceed those of Jackson by about 18 units in the sixth decimal place for each per cent of glucose, which, at a concentration of 25 per cent, is equivalent to less than 0.1 per cent glucose. They are still lower, however, than those for sucrose solutions of the same concentration.

Densities of Fructose Solutions. The following formulas have been established by Jackson and Mathews[17] for the densities of fructose solutions containing p per cent fructose by weight (in vacuo):

$$d_4^{20} = 0.99823 + 0.0038893\,p + 0.0000140\,p^2$$
$$d_4^{25} = 0.99708 + 0.0038557\,p + 0.0000139\,p^2$$

valid between 0 and 20 per cent, and

$$d_4^{20} = 0.99936 + 0.0037842\,p + 0.0000164\,p^2$$

valid from 20 to 70 per cent.

The table calculated by Jackson and Mathews from these formulas, and showing d_4^{20} and d_4^{25} of fructose solutions, in steps of 1 per cent sugar, is given in abridged form in Table XIV.

TABLE XIV

p	d_4^{20}	d_4^{25}
10	1.03853	1.03702
20	1.08162	1.07975
30	1.1276	1.1254
40	1.1769	1.17435
50	1.2295	1.2266
60	1.2853	1.2821
70	1.3444	1.3409

Below 49 per cent sugar the densities of fructose solutions are higher than those of sucrose solutions of the same concentration, at 49 per cent they are equal, and above that they become increasingly lower.

[17] *Bur. Standards J. Research*, **8**, 403 (1932).

The density d^t of fructose solutions found at temperature $t°$ C. may be corrected to 20° C. by the following formulas, for the range from 20° to 25° C.:

$$d^{20} = d^t + (0.000231 + 0.00000672\,p + 0.0000000224\,p^2)\,(t - 20)$$

which is valid between 0 and 20 per cent fructose, and

$$d^{20} = d^t + (0.0002145 + 0.00000795\,p + 0.0000000136\,p^2)\,(t - 20)$$

valid from 20 to 70 per cent fructose.

Jackson and Mathews also give a table to correct the readings obtained at temperatures between 18° and 28° C. with a Brix hydrometer, standardized for sucrose solutions at 20° C., but used to determine the concentration of fructose solutions.

Density tables for invert sugar solutions are not available as yet. If the density is midway between that for glucose and fructose, solutions containing up to 30 per cent of invert sugar have a slightly lower density than sucrose solutions of the same concentration.

Density and Mutarotation. Riiber[18] observed that the density of freshly prepared solutions of glucose, fructose, and other sugars changes upon standing. This phenomenon is due to the same cause as mutarotation, namely, the isomeric changes taking place. The volume of a solution of α-glucose or of β-fructose increases upon standing while that of β-glucose decreases. When equilibrium between the isomers is reached, the density becomes constant. In determining the density of solutions of mutarotating sugars it is therefore necessary to allow the solution to stand until the mutarotation is completed and the density has reached the equilibrium value.

Determination of Dissolved Solids by Use of Solution Factors. In the investigation of starch-conversion products the percentage of solids in 100 ml. of solution is frequently calculated from the specific gravity by means of a " solution factor." This method was introduced in 1876 by O'Sullivan,[19] who found that, when 10 g. of maltose or dextrin were dissolved at 60° F. (15.5° C.) to 100 cc., a solution of 1.0385 sp. gr. $(^{15.5°}_{15.5°})$ was obtained. Assuming that the percentage of dissolved substance is always proportional to the specific gravity of the solution (which is only approximately true), a solution containing 1 g. of maltose or dextrin in 100 cc. should have a specific gravity of 1.00385 at 15.5° C. A solution of specific gravity d should contain at 15.5° C. $\frac{1000(d - 1.000)}{3.85}$ g. of solids.

[18] *Ber.*, **56B**, 2185 (1923); **58B**, 737 (1925).

[19] *J. Chem. Soc.*, **1876**, 129.

Brown, Morris, and Millar,[20] determined the solution factors of a number of different sugars for a uniform specific gravity of 1.055 $\frac{15.5°}{15.5°}$ with the following results:

TABLE XV

SOLUTION FACTORS OF SUGARS AND STARCH CONVERSIONS

Anhydrous glucose	3.825
Anhydrous sucrose	3.859
Anhydrous invert sugar	3.866
Anhydrous fructose	3.907
Anhydrous maltose	3.916
Low starch conversion ($[\alpha]_D = +149.7$)	3.947
Medium starch conversion ($[\alpha]_D = +173.9$)	3.985
High starch conversion ($[\alpha]_D = +188.6$)	4.000
Dextrin	4.206

The solution factors of glucose, fructose, and maltose have been determined by Ling, Eynon, and Lane[21] with practically the same results as Brown, Morris, and Millar.

For ordinary purposes Brown, Morris, and Millar recommend the use of the sucrose factor 3.86. A comparison of the actual grams of sucrose per 100 cc. of solution with those calculated by means of this solution factor is given in the following table:

TABLE XVI

$d_{15.5°}^{15.5°}$	Sucrose in 100 cc. of Solution	Sucrose by Formula, $\frac{1000\,(d - 1.0000)}{3.86}$
	grams	grams
1.0039	1.00	1.01
1.0193	5.00	5.00
1.0386	10.00	10.00
1.0578	15.00	14.97
1.0770	20.00	19.95
1.0959	25.00	24.84
1.1149	30.00	29.76

Other sugars show similar variations in the solution factor with concentration. It is seen that the employment of solution factors, though sufficiently accurate for dilute solutions, is attended with considerable error upon liquids of high concentration. The factor 3.86 is not exactly the same for all sugars, so that this method of estimating solids is useful only for approximate purposes.

[20] *J. Chem. Soc.*, 71, 72 (1897).
[21] *J. Soc. Chem. Ind.*, 28, 730 (1909).

If the sugar solution is reduced to a uniform specific gravity of about 1.05 and a correction is made for the true density factor, the constant 3.86 can be employed without serious error. The correction is made by multiplying the results (percentages, specific rotation, reducing power, etc.) obtained by using the factor 3.86 by the value $3.86/F$, in which F is the true solution factor, according to Table XV, of the sugar in question.

Contraction in Volume of Sucrose and Water Mixtures. A phenomenon which has a most important bearing upon the specific gravity of solutions of sugars and other substances is that of contraction. If a definite quantity of sucrose, for example, is dissolved in a definite quantity of water, the volume of solution is always less than the sum of the volumes of sucrose and water taken. The same is also true, but to a less extent, of the mixture of sucrose solutions of different concentration and of sucrose solutions with water. The phenomenon of contraction in volume during solution of sucrose and water has long been known. It was first observed by Réaumur and Petit le Médecin in 1733, and has been repeatedly studied by many subsequent observers.[22] The extent of this contraction has been variously estimated. If x is the percentage of dissolved sucrose, the change in volume v according to Brix[23] is represented by the equation

$$v = 0.0288747\,x - 0.000083613\,x^2 - 0.0000020513\,x^3$$

Scheibler[24] gives the equation

$$v = 0.0273731\,x - 0.000114939\,x^2 - 0.00000158792\,x^3$$

according to which the maximum contraction is 0.8937 cc. for 55.42 g. sucrose and 44.58 g. water at 17.5° C. Gerlach gives the maximum contraction as 0.9946 cc. for 56.25 g. sucrose and 43.75 g. water, and Ziegler[25] as 0.9958 cc. for 56 g. sucrose and 44 g. water.

According to Matthiessen and others,[26] the maximum contraction is reached at about 40 per cent sucrose; beyond this there is a decrease until at 60 per cent sucrose the contraction is 0; with concentrations above 60 per cent sucrose there is an expansion in volume. This view of the question is due, according to Plato,[27] to the mistaken idea that

[22] Olizy (*Bull. assoc. chim. sucr. dist.*, 27, 60) claims to have demonstrated that no contraction takes place during the solution of sucrose in water.

[23] *Z. Ver. deut. Zucker-Ind.*, 4, 308 (1854).

[24] *Neue Zeitschrift*, 25, 37 (1890).

[25] *Oesterr.-ungar. Z. Zuckerind. Landw.*, 12, 760 (1883).

[26] Lippmann, "Chemie der Zuckerarten." p. 1081, 1904.

[27] *Z. Ver. deut. Zucker-Ind.*, 50, 1098 (1900).

dissolved sucrose has the same specific gravity as the crystallized solid (1.59103 $\frac{15°}{15°}$ for chemically pure powdered sucrose, 1.5892 $\frac{15°}{15°}$ for chemically pure sucrose crystals). If we take Plato's calculated value for the specific gravity of dissolved sucrose in aqueous solution, 1.55626, the following results (Table XVII) are obtained, which are in close concord-

TABLE XVII

CONTRACTION IN VOLUME OF SUCROSE-WATER MIXTURES

Per Cent Sucrose	Contraction of Mixture		Per Cent Sucrose	Contraction of Mixture	
	For 1 kg.	For 1 liter		For 1 kg.	For 1 liter
	ml.	ml.		ml.	ml.
0	0.0	0.0	55	10.3	13.4
5	1.5	1.5	60	10.3	13.7
10	2.9	3.0	65	10.0	13.7
15	4.2	4.5	70	9.6	13.4
20	5.4	6.0	75	8.8	12.6
25	6.5	7.4	80	7.7	11.5
30	7.5	8.7	85	6.2	9.8
35	8.4	9.9	90	4.6	7.5
40	9.1	11.0	95	2.4	4.3
45	9.7	12.0	100	0.0	0.0
50	10.1	12.8			

ance with those of Gerlach and Ziegler. The apparent change in specific gravity of dissolved sucrose is due to the phenomenon of contraction, for which no satisfactory explanation has yet been offered, although it is probably connected with the fact that sucrose exists in solution not as such, but in the form of a hydrate or hydrates.

TABLE XVIII

CONTRACTION IN VOLUME OF A 60 PER CENT SUCROSE SOLUTION AND WATER

A Solution Taken	*B* Volume of Solution, 17.5°	*C* Water Taken	*D* Volume of Water, 17.5°	*E* Volume before Mixing, $B + D$	*F* Volume after Mixing	Contraction (E–F)
grams	ml.	grams	ml.	ml.	ml.	ml.
0	0.000	100	100.126	100.126	100.126	0.000
5	3.876	95	95.120	98.996	98.840	0.156
10	7.752	90	90.113	97.865	97.682	0.183
20	15.504	80	80.101	95.605	95.372	0.233
40	31.008	60	60.076	91.084	90.789	0.295
50	38.760	50	50.063	88.823	88.521	0.301
60	46.512	40	40.050	86.562	86.273	0.289
80	62.016	20	20.025	82.041	81.845	0.196
90	69.768	10	10.013	79.781	79.670	0.111
95	73.644	5	5.006	78.650	78.595	0.055
100	72.526	0	0.000	72.526	72.526	0.000

The effect of mixing sucrose solutions and water is shown in Table XVIII, which gives the calculated contraction of mixtures of 60 per cent sucrose solutions with water to make 100 g.

The Specific Gravity of Impure Sugar Solutions. While the application of specific-gravity tables established for sucrose to the estimation of dissolved substance in not too concentrated solutions of other sugars and carbohydrates is fairly accurate, their use for impure sugar solutions may lead to serious errors, owing to the fact that the percentage of dissolved impurities for the same specific gravity differs from the corresponding percentage of sucrose. The errors resulting from this cause may be seen in Table XIX, which gives the concentra-

TABLE XIX

CONCENTRATIONS OF AQUEOUS SOLUTIONS OF ORGANIC AND INORGANIC COMPOUNDS COMPARED WITH THOSE OF SUCROSE AT 15° C. FOR THE SAME SPECIFIC GRAVITY

Specific Gravity	Sucrose	Tartaric Acid	NaK Tartrate	K_2CO_3
	per cent	per cent	per cent	per cent
1.0039	1	0.87	0.57	0.43
1.0078	2	1.73	1.14	0.86
1.0118	3	2.62	1.71	1.29
1.0157	4	3.49	2.28	1.72
1.0197	5	4.40	2.87	2.15
1.0402	10	8.67	5.87	4.40
1.0833	20	17.52	12.16	9.00
1.1296	30	26.29	18.38	13.78
1.1794	40	35.33	24.73	18.72
1.2328	50	44.22	31.10	23.76

tions of sucrose, tartaric acid, sodium potassium tartrate, and potassium carbonate for different specific gravities. When the specific gravity is determined after dilution with a definite amount of water, as is necessary with very thick sirups, the error in estimation of dissolved substance is still further intensified, owing to the difference in contraction between sugar and dissolved impurities in aqueous solution. This can be seen by reference to Table XIX; it is also shown in Table XX, which gives the calculated differences in contraction obtained by diluting solutions of sucrose, tartaric acid, sodium potassium tartrate, and potassium carbonate with water to reduce degrees Brix from 50 to 10.

Similar figures for molasses diluted with an equal quantity of water are given in Table XXIII. Additional comparisons showing the differences between true dry substance and dry substance as calculated from specific gravity are given for a number of compounds in Table XXVII.

TABLE XX

CONTRACTION ON DILUTING MIXTURES OF SOLUTIONS OF ABOVE SUBSTANCES WITH WATER TO REDUCE DEGREES BRIX FROM 50 TO 10. SOLUTION TAKEN, 100 G., 1.2328 SP. GR., OR 81.49 CC. SPECIFIC GRAVITY AFTER DILUTION, 1.0402. TEMPERATURE 15° C.

Substance	Dissolved Substance, per cent		Water Added		Volume before Mixing	Actual Volume after Mixing	Contraction ($E-F$)
	Before Dilution A	After Dilution B	$\left(\frac{100\,A}{B}\right)-100$ C	D	$E=(D+81.49)$	$F=\frac{(100+C)}{(1.0402)}$	
			grams	cc.	cc.	cc.	cc.
Sucrose	50.00	10.00	400.00	400.34	481.83	480.67	1.16
Tartaric acid	44.22	8.67	410.04	410.38	491.87	490.32	1.55
NaK tartrate	31.10	5.87	429.81	430.17	511.66	509.34	2.32
K_2CO_3	23.76	4.40	440.00	440.37	521.86	519.13	2.73

METHODS OF DETERMINING SPECIFIC GRAVITY OF SUGAR SOLUTIONS

In the estimation of dissolved sugars by means of specific gravity, the temperature of the laboratory is not always the same as that prescribed by the table. It is then necessary either to bring the solution to the required temperature by artificial means or else to apply a fixed correction from a conversion table. The correction method is the more convenient and for ordinary purposes is sufficiently exact; however, where great accuracy is required the determination must be conducted under absolutely the same temperature conditions as specified in the tables.

Specific-Gravity Bottle or Pycnometer. The most accurate method for the determination of specific gravity is the direct comparison of the weights of equal volumes of water and sugar solution. In this method some form of specific-gravity bottle or pycnometer is used, various types of which are shown in Figs. 20 to 23.

Before using the instrument the pycnometer is calibrated by determining the weight of distilled water which it contains at the temperature of comparison. The bottle is first thoroughly cleaned by means of dilute caustic soda and hydrochloric acid; it is then washed with distilled water and dried in an air bath. The thermometer stem of a pycnometer should never be warmed beyond the limit of graduation, which is frequently only 40° C.; otherwise the expansion of the mercury may break the instrument. After drying and cooling the pycnometer is weighed. The bottle is next filled with distilled water, recently boiled and cooled to expel dissolved air. The temperature

adjustment is best effected by filling the bottle with water a degree or so lower than the temperature desired; the stopper is then inserted, taking care to prevent the introduction of air bubbles, and the bottle placed in a bath of water kept exactly at the desired temperature. After about 10 minutes, or as soon as the thermometer of the instrument has risen to the right degree, the excess of water, exuding from the stem, or above the graduation mark, is removed with a thin piece of filter paper, the cap is fitted, and the bottle wiped perfectly dry and reweighed. The increase in weight is the water capacity of the bottle at the desired temperature. The process is repeated and the average of several determinations used as a constant in all subsequent work.

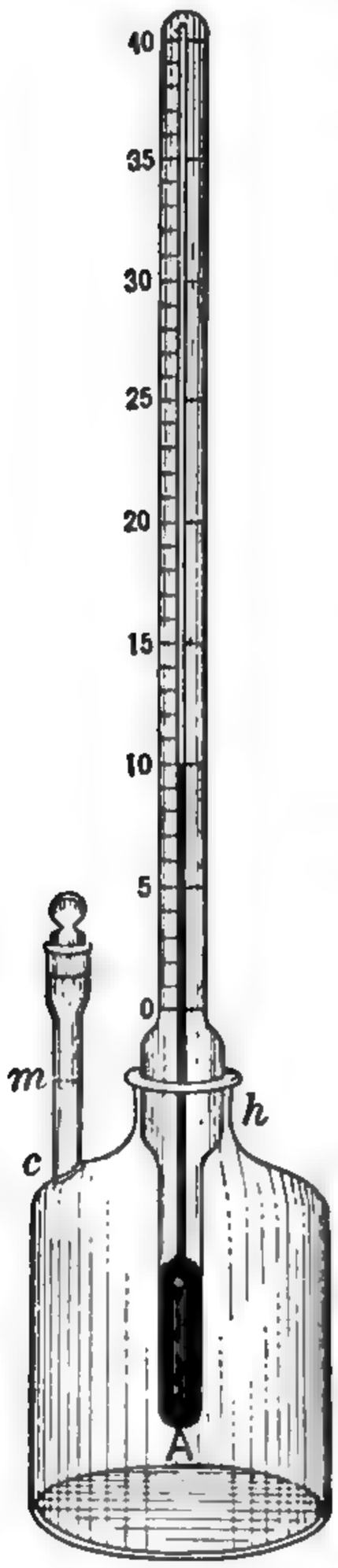


FIG. 20. Specific-gravity bottle with thermometer.

The pycnometer, after redrying or rinsing repeatedly with the liquid to be examined, is next filled with the sugar solution (observing the same precautions as to temperature as before) and reweighed. The weight of solution divided by the water capacity of the bottle gives the apparent specific gravity.

Since 20° C. has been adopted as the standard temperature[28] for all processes of sugar analysis, it is best to make the determination of specific gravity when possible at this temperature. At the seventh session of the International Commission for Uniform Methods of Sugar Analysis, held in New York in 1912, it was agreed that "in specific-gravity determinations of aqueous solutions the specific gravity obtained at the normal temperature shall be referred to the density of water at 4° C. and to vacuum." The necessary calculations may be made by the following formula:

$$s = \frac{m}{w}(Q - d) + d$$

where s is the true density of the solution at the temperature at which it has been weighed, referred to water at 4° and to vacuum; m is the

[28] At the sixth session of the International Commission for Uniform Methods of Sugar Analysis (London, May 31, '909) it was "voted unanimously to accept a single specific-gravity table as stand. :d, at the temperature of 20° C., which is to be based upon the official German table. From this, other tables may be calculated at other temperatures, for instance, at 15° C., 17.5° C., 30° C., etc."

weight of the solution, determined in air; w the weight of an equal volume of water, determined in air; Q the density of the water at the temperature at which it has been weighed; and d the mean density of

FIG. 21.

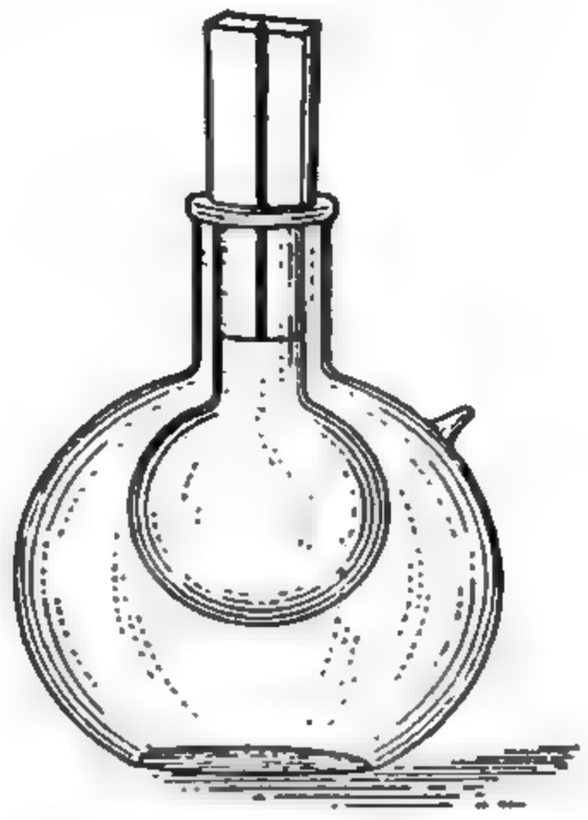


FIG. 22.

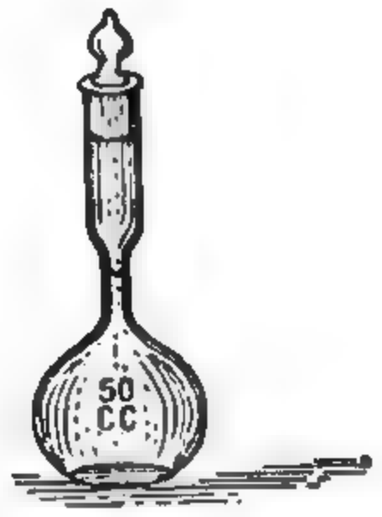


FIG. 23.

Types of specific-gravity bottles.

air (0.00120). If the weighings have all been carried out at the normal temperature of 20° C., substitution of the density of water at 20° C. (0.99823) leads to the simplified equation:

$$s = \frac{0.99703\ m}{w} + 0.0012$$

or in other words, the apparent specific gravity $\frac{20°}{20°}$ is multiplied by 0.99703, and 0.0012 is added to the product.

Instead of calculating the true density, and then finding from Plato's table the corresponding percentage of sucrose, the latter may be ascertained directly from the apparent specific gravity by the use of Table 3 in the Appendix, provided that the barometric pressure does not differ appreciably from 760 mm. of mercury.

If the temperature of the laboratory is much above that of adjustment, the specific-gravity bottle and contents must remain at rest until they acquire the surrounding atmospheric temperature; otherwise moisture will condense upon the instrument and interfere with the weighing. It is needless to add that the cap of the bottle must be sufficiently tight to prevent leakage of liquid displaced by expansion through increase of temperature. Pycnometers whose stems are to be filled to mark and hence allow room for expansion, as Fig. 20, are generally to be preferred. For certain kinds of work (as for densities of very dilute sugar solutions) Sidersky[29] recommends Boot's pycnometer

[29] "Les densités des solutions sucrées," p. 17.

(Fig. 22), which, having a double wall with vacuum, keeps the temperature of the solution constant for a long time.

For highly concentrated sugar solutions, such as molasses, massecuites, or other viscous substances, the method must be somewhat modified, if the specific gravity of the undiluted material is desired. These products usually contain occluded air and other gases which must first be removed because they depress the specific gravity of the material. In the method prescribed by the United States Treasury Department[30] for determining the density of molasses a special 100-ml. volumetric flask, with a neck of approximately 8-mm. inside diameter, is used as a pycnometer. The exact volume of the flask must be determined by calibration. The flask is weighed empty and then filled with molasses by means of a long-stem funnel reaching below the graduation mark, until the level of the molasses is up to the lower end of the neck of the flask. The funnel is withdrawn carefully so that it does not come in contact with the neck of the flask. The weight of the flask and molasses is determined. Then water is added almost up to the graduation mark, by running it down the side of the neck to prevent mixing with the molasses. The flask is allowed to stand for several hours or overnight to permit the escape of bubbles. It is then placed in a water bath at 20° C. for a sufficient time for it to reach the temperature of the bath, made to volume at that temperature with water, and reweighed.

The calculation of the density is illustrated by the following example:

A, weight of flask empty	37.907 g.
B, weight of flask and molasses	167.148 g.
C, weight of flask, molasses, and water	174.711 g.
C–*B*, weight of water	7.563 g.
B–*A*, weight of molasses	129.241 g.

To find the volume of the molasses, the weight of the water is divided by the weight of 1 ml. of water, weighed in air at 20° C.

$$\frac{7.563}{0.99718} = 7.584 \text{ ml.}$$

This volume is deducted from the volume of the flask, which has been found to be 100.060 ml.

The volume of the molasses is 100.060 − 7.584 = 92.476 ml.

[30] U. S. Customs Regulations, 1931, Art. 762.

Next the weight of the molasses, found in air with brass weights, is reduced to vacuo, by calculating the buoyancy correction to be applied. Assuming a density of 8.4 for the brass weights, the volume of the weights is 129.241 ÷ 8.4 = 15.4 ml. Deducting this volume from the approximate volume of the molasses, 92.5 ml., we find an air displacement of 77.1 ml. This is now multiplied by the weight of 1 ml. of air at 20° C. and 760 mm. pressure, 0.0012 g., giving 77.1 × 0.0012, or 0.093 g., as the buoyancy correction to be added to the weight of the molasses: 129.241 + 0.093 = 129.334 g., weight of the molasses in vacuo. This, divided by the volume of the molasses, 92.476 ml., gives a true density $\frac{20°}{4°}$ of 1.3986, which according to Plato's table corresponds to 78.0 Brix.

The calculation may be simplified by computing the apparent specific gravity $\frac{20°}{20°}$ directly from weights in air. The water capacity of the flask at 20° C. is obtained by multiplying the volume, 100.060 ml., by 0.99718, giving 99.778 g. The weight of the water above the molasses, $C - B$, or 7.563 g., is deducted, and the result, 92.215, is divided into the weight of the molasses, $B - A$: 129.241 ÷ 92.215 = 1.4015, which according to Table 3 in the Appendix corresponds to 78.0 Brix, the same as before.

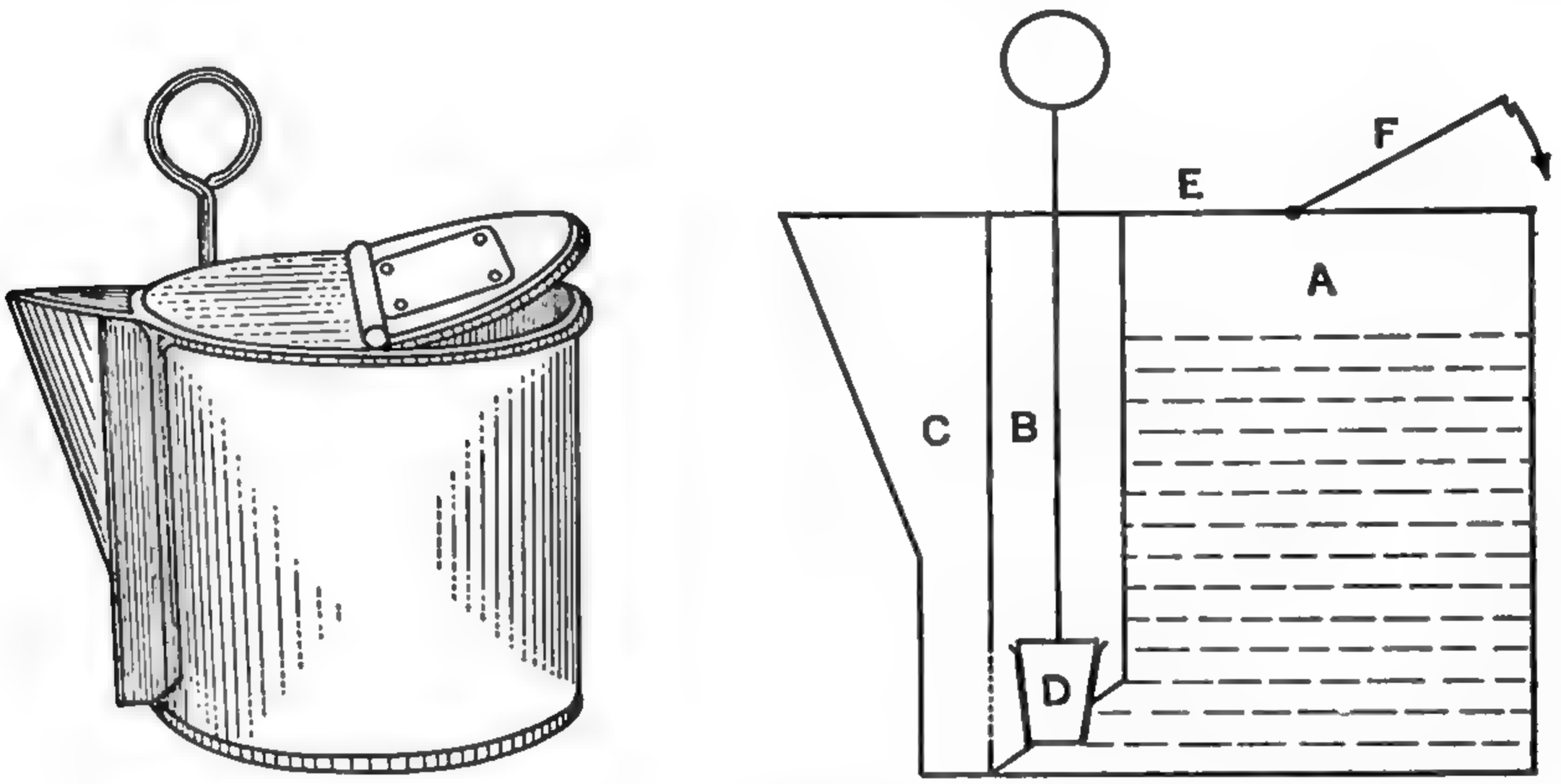
If the molasses is not too viscous the gas bubbles rise to the surface upon standing at room temperature, as prescribed in this method, but with heavy molasses even prolonged standing does not produce a gas-free product. This makes it necessary to promote the escape of bubbles by other means.

A pycnometer with rather wide neck, of the form in Fig. 23, may be used, and filled nearly to the mark with the hot material to be examined. The flask is placed for a short time in an oil or salt-water bath, the boiling point of which is sufficiently high to keep the material in a liquid condition. After the flask is cooled to 20° C. and weighed, the space between the substance and the graduation mark is filled with distilled water and the flask reweighed. The calculation is made in the same way as in the example given before.

Reich[31] has modified the above method by filling the pycnometer to mark directly from a burette divided into 0.05 ml. and noting the volume of water added. If the burette has 50 instead of 0 as the top graduation, the actual cubic centimeters of molasses, etc., in the pycnometer is read off directly when the instrument is calibrated to hold exactly 50 ml. This of course obviates a second weighing of the pycnometer, and, while not as accurate as the method of weighing, is sufficiently close for many purposes.

[31] *Deut. Zuckerind.*, 34, 38 (1909).

In a method very generally used in the beet-sugar industry the molasses is heated in a hot-water funnel provided with a glass rod the lower end of which is ground into the upper end of the funnel stem. The funnel is filled with the cold molasses, while the glass rod is in place, and the water in the jacket is heated to a temperature high enough to permit the free escape of the gas bubbles. A scum forms on the surface, protecting the molasses to some extent from evaporation. As soon as a sharp line of separation is noted between the liquid and the scum, the funnel is allowed to cool to about 30° C. The glass rod is



(*Reproduced from Z. Zuckerind. Böhmen, 35, 241.*)

FIG. 24. Urban's heating vessel for molasses.

carefully lifted, and the first portion of the effluent, which contains the sand and other heavy sediment, is discarded. The pycnometer is then filled and the determination completed as already described.

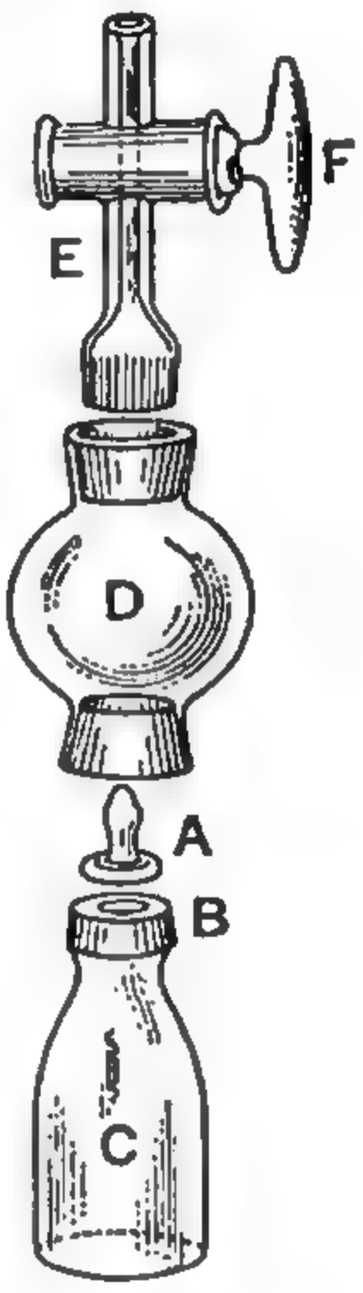
Urban[32] has devised a heating arrangement, shown in Fig. 24, by which evaporation may be reduced to a minimum. The apparatus is made of sheet copper. The molasses is run into compartment *A*, and the cover *F* is closed. The entire vessel is placed in a hot-water bath to such a depth that the surface of the water is level with that of the molasses. As soon as the molasses is free from gas, stopper *D* is slowly lifted; the molasses flows into compartment *B* and from there through a sieve into compartment *C*, from which the pycnometer is filled.

Even with this apparatus there is some danger of evaporation, and the specific gravity may be found too high.

[32] *Z. Zuckerind. Böhmen,* **35, 239 (1910/11).**

Newkirk's Pycnometer for Molasses. Newkirk[33] has devised a pycnometer which, by its attachment for removing dissolved and occluded gases, is especially adapted to determining the specific gravity of molasses and other sugar-containing liquids of high density. The apparatus is shown in Fig. 25.

It consists of a bottle, *C*, fitted with an enlargement at the top, *B*, ground optically flat and closed off by another optical flat, *A*. An expansion chamber, *D*, is ground to the bottle and fitted with a vacuum connection, *E*. To avoid loss of water due to evaporation under reduced pressure, the connecting tube is fitted with a stopcock, *F*, so that when the proper vacuum has been reached the apparatus can be closed off from the vacuum source. With this provision the volume to be filled with water vapor is very small and the amount of water evaporated will be negligible. The bottle is so shaped as to have a smooth gradual slope to the top, so that the bubbles will rise with the least effort to the expansion chamber. It has thick walls over the neck, so that it can be readily handled without changing the temperature or volume by heat transmitted to the flask by the fingers. The joints of the expansion chamber, vacuum connection, and stopcock are ground to an accurate fit. Since it is unnecessary to employ a vacuum with a pressure lower than the vapor pressure of molasses at room temperature, it is entirely practicable to utilize the sample under test to lubricate and seal all the ground joints. Needless to say, this is an important advantage.



(Courtesy of Eimer & Amend.)

FIG. 25. Newkirk's pycnometer for molasses.

In using the pycnometer, the expansion chamber, after lubrication of all joints with molasses, is placed on the bottle. The molasses to be analyzed is flowed into the bottle and into the expansion chamber until the latter is about one-third full. The vacuum line is then connected and the pressure reduced until the gas expands to visible bubbles. The apparatus is immediately closed off by turning the stopcock, *F*, and the whole placed in the thermostat for accurate work or in the balance case for control work. When all the bubbles have collected in the expansion chamber and the temperature has reached equilibrium, the volume is fixed with the plate after removing the expansion chamber. It is then wiped and weighed.

The densities are determined by correcting the weights to vacuo and comparing to the weight of an equal volume of water at 4° C. in vacuo.

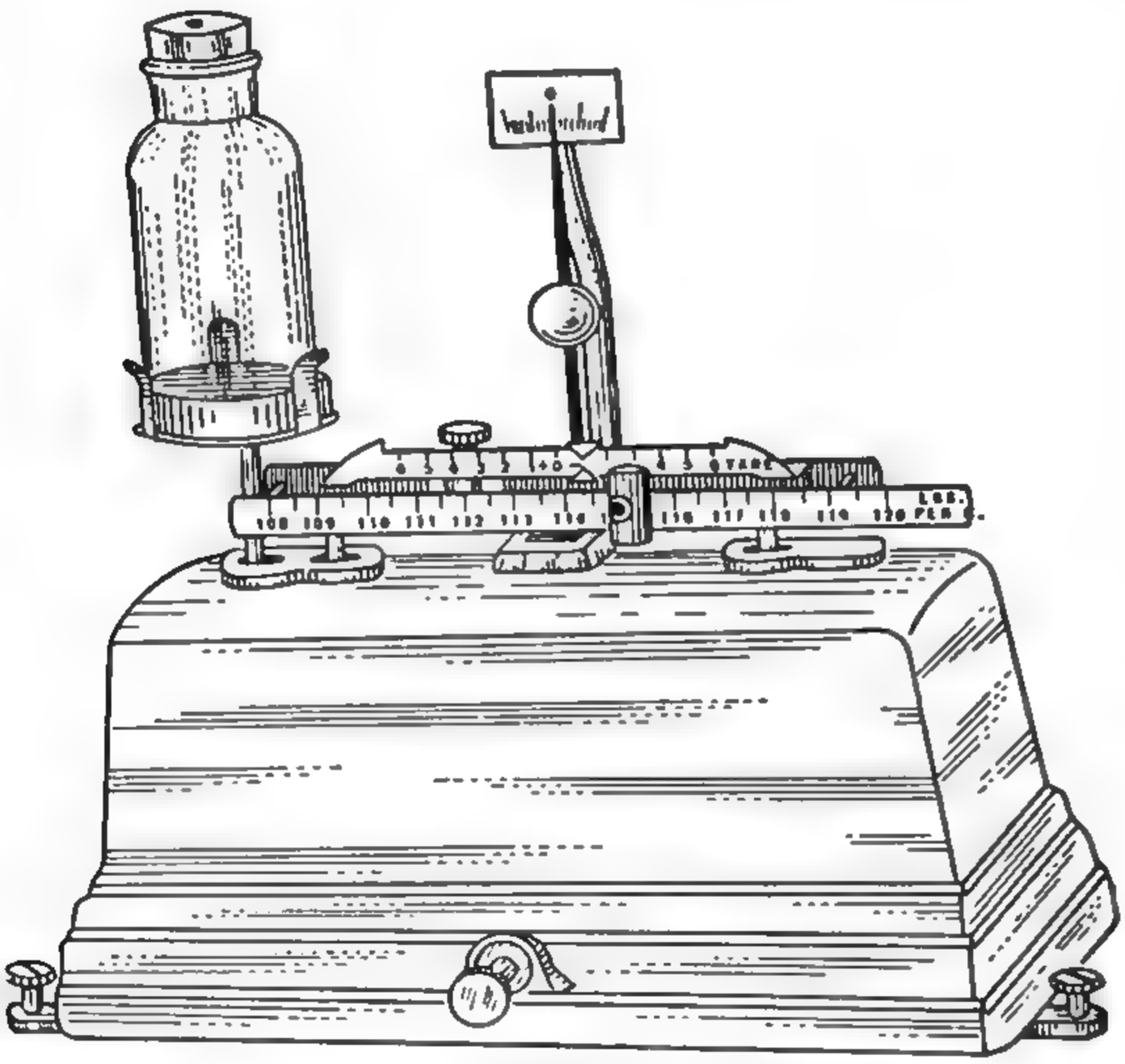
Although the Newkirk method has the advantage that the air is eliminated more rapidly and more completely than by allowing the

[33] *Bur. Standards Tech. Paper* 161, 1920.

molasses to stand at room temperature, as prescribed in the United States Treasury method (p. 62), Hayes[34] has reported a molasses which after being kept under vacuum for months still showed considerable gas in suspension. In contradistinction to the heating method, the vacuum method is likely to give low results.

The weight per gallon, at 20° C., corresponding to the specific gravity of a sirup or molasses may be found by referring to Table 3 in the Appendix; Table 5 may be used for converting the pounds per gallon at one temperature into those at another temperature, between 10° and 30° C.

Bastone's Torsion Balance for Molasses.[35] For the rapid estimation of the pounds per gallon of molasses Bastone has devised a convenient torsion computing balance, Fig. 26, which is provided with two beams. The bottle for holding the molasses is of special design, with a wide neck permitting the bottle to be easily filled, emptied, and cleaned, and with an accurately fitting perforated stopper. The capacity is 100 ml. at 20° C. The empty bottle is first counterbalanced by means of the back or tare beam. It is next filled with the molasses and placed on the holder, and the pointer of the scale is brought to equilibrium by means of the slide weight on the front or recording beam. The position of the slide indicates directly pounds per gallon in air of the molasses. The same precautions for temperature, subsidence of foam, and escape of occluded gases must be observed with the torsion balance as with the pycnometer.



(*Courtesy of Christian Becker, Inc.*)

FIG. 26. Bastone's molasses balance.

Raw juices from the cane or beet also contain much occluded air which must be removed before the specific gravity is determined. With

[34] *Proc. Ann. Congr. S. African Sugar Tech. Assoc.*, 6th Meeting, p. 9, 1932.

[35] For a fuller description of Bastone's torsion balance for molasses see *Bur. Standards Tech. Paper* 345, by Snyder and Hammond.

cane juices it is usually sufficient to allow them to stand in a tall cylinder for about half an hour. But raw beet juices usually produce so much foam that it is preferable to use vacuum for de-airing. The juice is placed in a bottle with an outlet cock near the bottom, the mouth of the bottle is connected with a vacuum pump, and when all the air bubbles have risen to the surface, the juice is drawn off through the stopcock at the bottom.

Browne's Specific-Gravity Bottle and Dilatometer. For determining the changes in density and volume which sugar solutions undergo with varying conditions, Browne[36] has devised a combination specific-gravity bottle and dilatometer (Fig. 27). The apparatus consists of a narrow tubular body, *B*, holding about 30 ml., connected at the bottom with a graduated capillary tube, *A*, and contracted at the top to an opening at *O*. *O* is made slightly funnel-shaped and is carefully ground on its inner surface so as to receive the thermometer *T*, which is also ground above its scale so as to fit perfectly tight after insertion. The displacement of the thermometer is about 7 ml., which leaves a capacity of about 23 ml. for the instrument after stoppering.

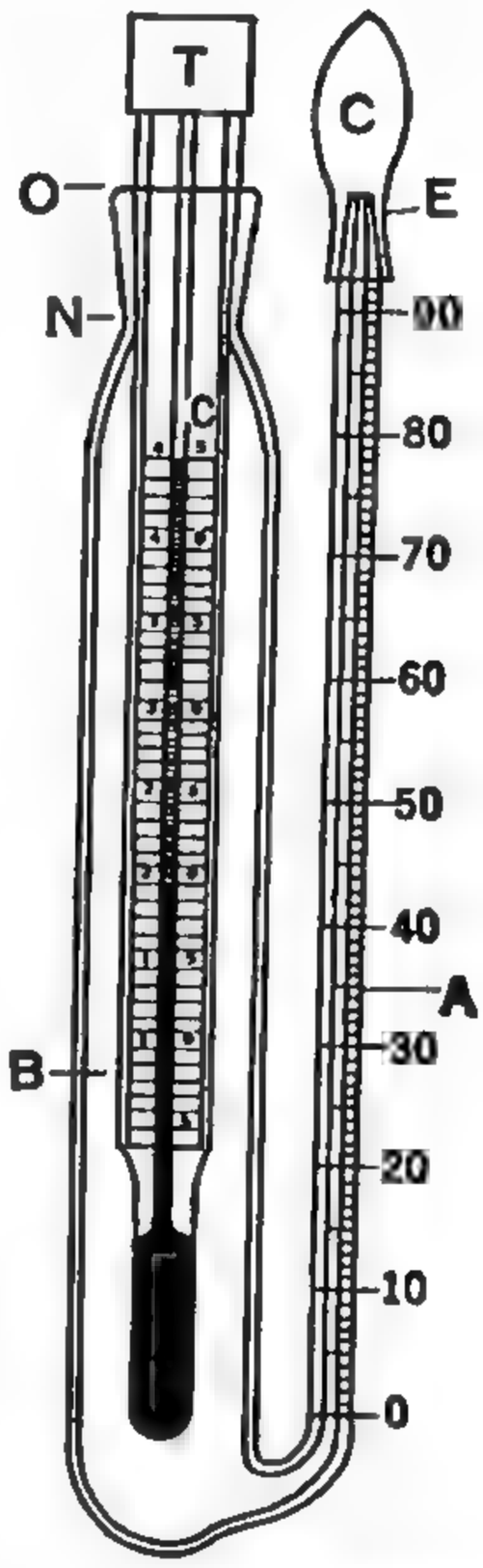


FIG. 27. Browne's specific-gravity bottle and dilatometer.

The end of the capillary tube at *E* is ground and fitted to a small cap, *C*. The scale upon which the changes of volume are measured is graduated so that 1 division equals 0.001 ml.; by means of a magnifying glass, readings can be made to 0.0001 ml.

A few weighings of the instrument, after filling with air-free distilled water to different points of the scale at different temperatures, are sufficient for constructing a table of water constants for each scale division and temperature. The ground-glass surfaces should be lightly coated with vaseline to prevent all possibility of loss from evaporation. The instrument, when filled with water and stoppered, should show no loss in weight after a week's standing. In making weighings the bottle may be placed in a support, or it may be attached to the hook of the balance beam by means of a loop of wire wound about the neck at *N*.

For determining specific gravities the method of operation is the same as with an ordinary pycnometer. If it is desired to determine the

[36] *J. Am. Chem. Soc.*, 35, 955 (1913).

specific gravities of a solution at different temperatures, one filling of the instrument and one weighing only are required. After the apparatus is filled the meniscus in the capillary tube is adjusted by means of a thin, tightly wound strip of filter paper to a convenient position upon the scale and the whole is weighed. The instrument is then placed in a constant-temperature chamber (such as an incubator), and as soon as the thermometer and meniscus readings remain constant the observations are noted. By raising or lowering the temperature and noting the changes in position of the meniscus, the specific gravities and coefficients of expansion or contraction may be readily calculated. The position of the meniscus is easily affected by very slight changes in temperature, so that the instrument is best handled by placing it in a stoppered glass cylinder.

The apparatus can also be used for measuring the contraction which sugar solutions undergo during hydrolysis or inversion. The instrument is filled with the freshly prepared solution of sugar and hydrolyzing agent (such as acid or invertase or other enzyme) at the desired temperature of the experiment, the meniscus is set at some division high upon the scale, and the whole is weighed. After placing in the constant-temperature chamber the progressive changes in volume and specific gravity are easily followed. Since 100 ml. of a 1 per cent sucrose solution undergoes a contraction during inversion of very closely 0.02 ml., the approximate percentage of sucrose may be estimated by noting the change in volume which a solution undergoes upon treatment with invertase or mineral acids. While this method of estimating sucrose has not the same degree of accuracy as calculations based upon determinations of polarizing or copper-reducing power before and after inversion, the method may be used in certain cases as a confirmatory one.

DETERMINATION OF SPECIFIC GRAVITY BY DISPLACEMENT METHODS

A second method for determining the specific gravity of sugar solutions is based upon the well-known principle of Archimedes, that a body immersed in a liquid loses the same weight as that of the volume of liquid displaced. It is therefore only necessary to compare the losses in weight which the same body undergoes in water and in a given solution, in order to determine the specific gravity of the solution. The process may be carried out in a variety of ways; a common method is by means of the analytical balance.

A sinker of heavy glass, or a bulb of glass containing mercury, is attached to a silk thread and weighed first in air, then in distilled water,

and finally in the sugar solution. The method of conducting the weighing is shown in Fig. 28.

The method of calculation is shown by the following example:

A, weight of sinker in air = 25.345 g. at 20° C.
B, weight of sinker in water = 22.302 g. at 20° C.
C, weight of sinker in sugar solution, = 21.504 g. at 20° C.

Specific gravity of sugar solution, $S = \frac{A - C}{A - B} = \frac{3.841}{3.043} = 1.2622\ {}^{20°}_{20°}$ in air.

To convert to true density with reference to weights in vacuo, the formula given on p. 60 is used.

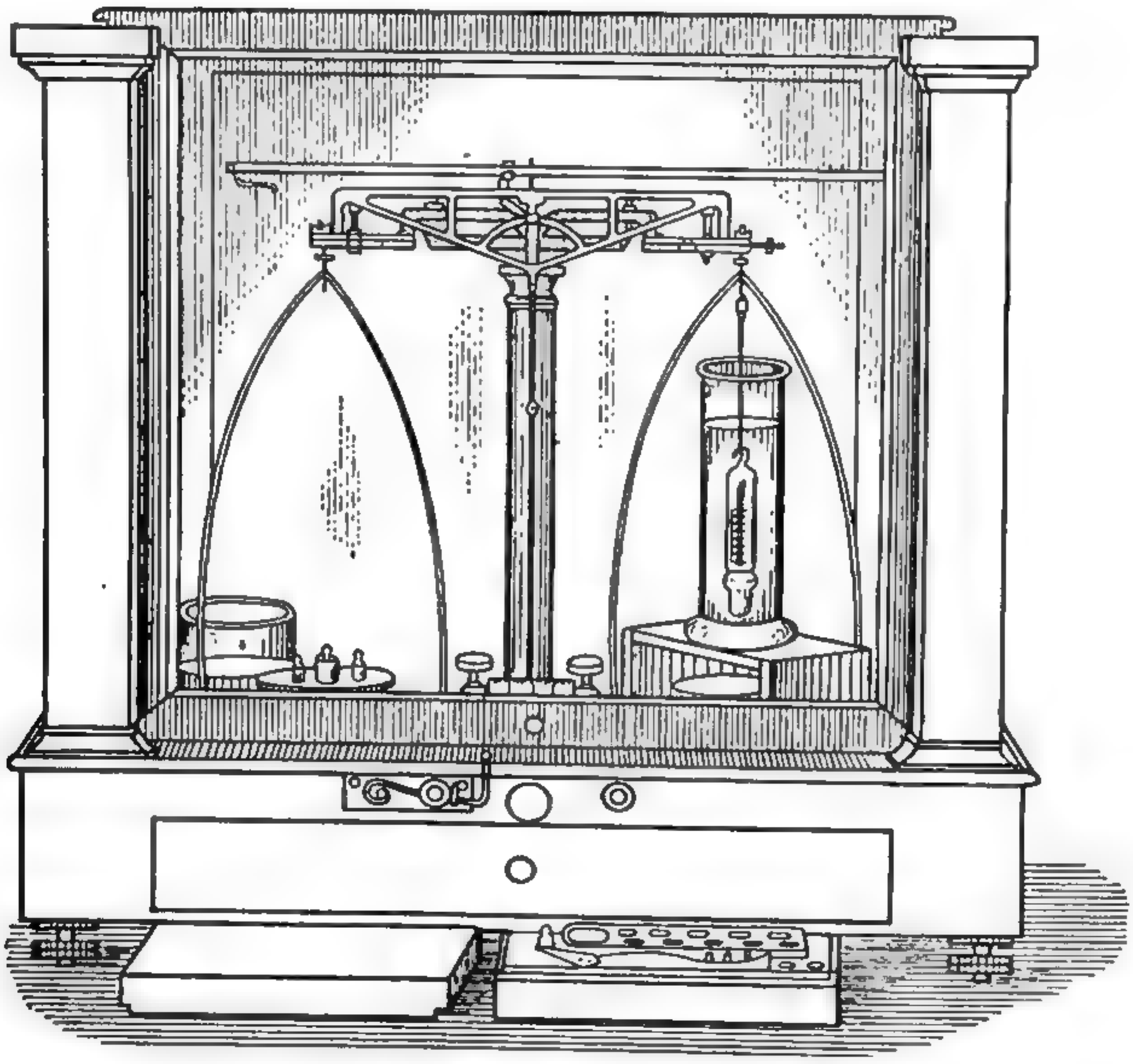


FIG. 28. Determination of specific gravity by means of analytical balance.

Mohr's Specific-Gravity Balance. The specific-gravity balance of Mohr, as improved by Westphal, and hence frequently called the Westphal balance, makes use of the principle of the sinker described in the previous section. The construction and operation of the balance are best understood from Fig. 29. The beam AC of the balance is pivoted at B and between the pivot and point of suspension C is divided by notches into 10 equal parts. The distance between each two divisions of the beam is ordinarily made exactly 1 cm. The balance, as usually

supplied, has a specially constructed thermometer sinker (Reimann's thermometer body) which by careful grinding of the lower end is made to displace exactly 5 g. of distilled water at 15° C. The sinker is attached by means of a fine platinum wire to the brass hanger *H*, the combined weight of sinker, wire, and hanger being made to equal exactly 15 g. Before using, the balance is first adjusted by hanging the sinker from the arm and regulating the screw *S* until, when the beam

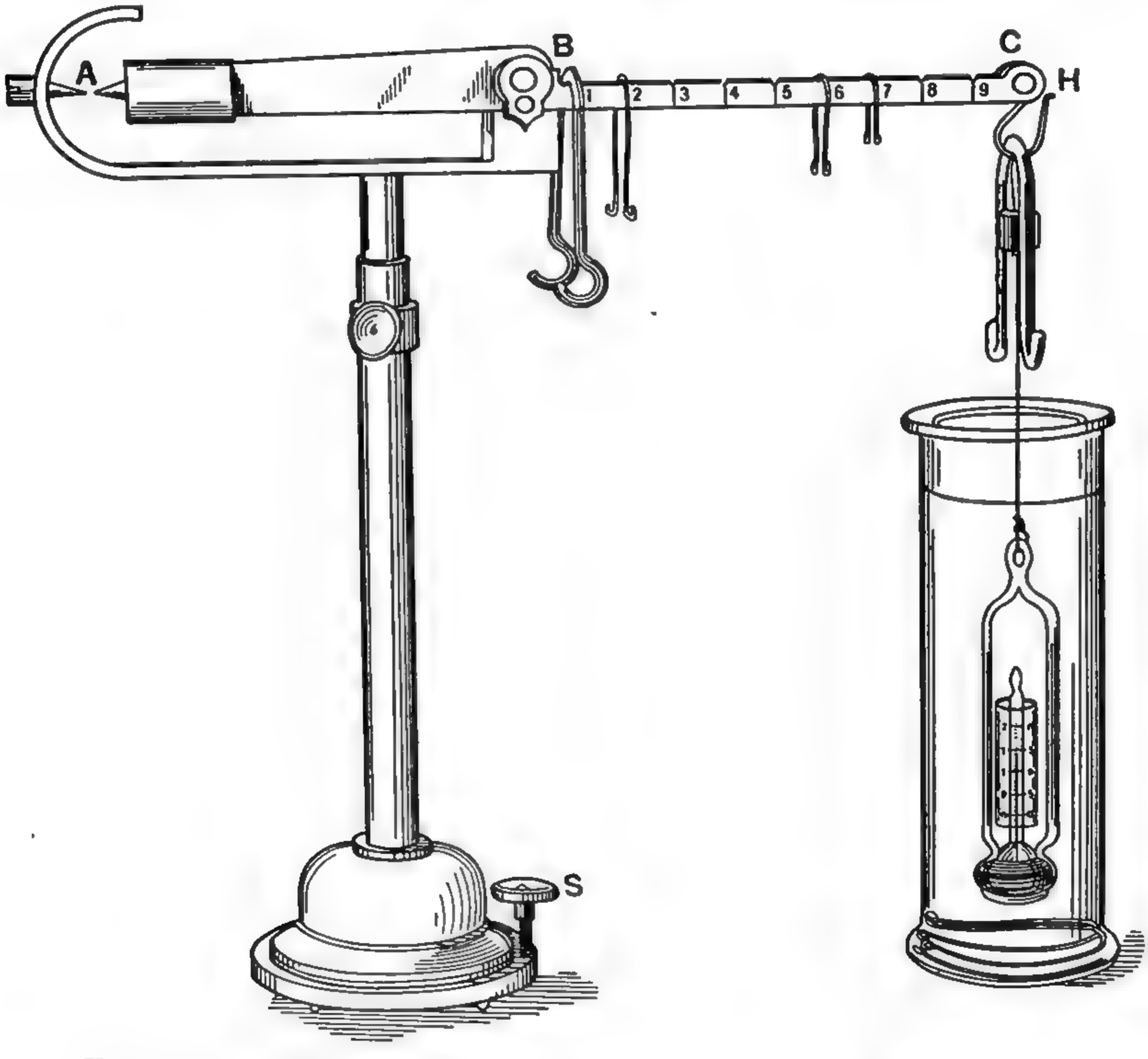


FIG. 29. Mohr's specific-gravity balance (indicating 1.1267 sp. gr.)

is at rest, the pointers of the arm and support at *A* exactly coincide. If the sinker is now submerged in distilled water at 15° C., it will require 5 g. at the point of suspension *C* to restore equilibrium. The standard weight for Reimann's thermometer body is therefore 5 g., and in determining the specific gravity of solutions heavier than water this weight must always be hung from the point *C*. To obtain the decimal figures of the specific gravity, weights are added to the notches on the beam until the pointers indicate equilibrium. The first decimal figure is

obtained by means of a duplicate 5-g. weight, which is moved from notch to notch on the beam until the correct decimal is secured; the second decimal figure is obtained by means of a 0.5-g. weight, the third decimal figure by a 0.05-g. weight, and the fourth decimal figure by a 0.005-g. weight. The specific gravity is then read from the scale divisions of the beam in the order of the diminishing weights. The method of reading is easily understood from Fig. 30.

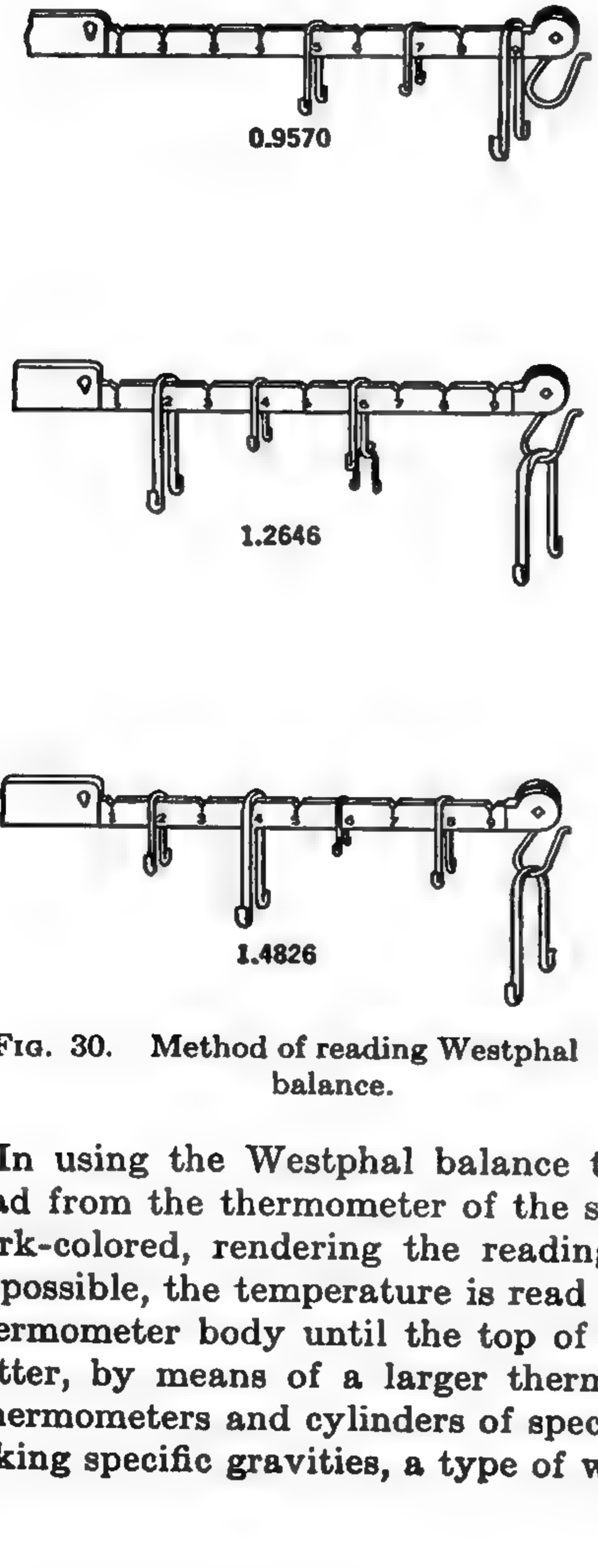


FIG. 30. Method of reading Westphal balance.

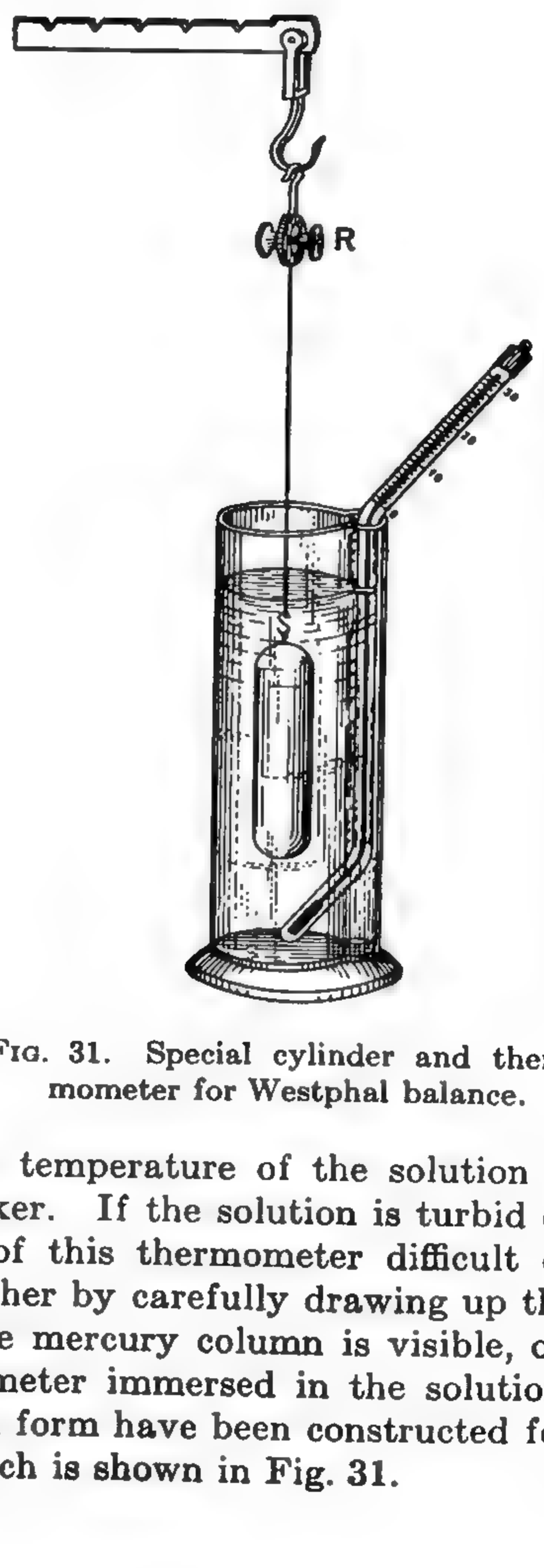


FIG. 31. Special cylinder and thermometer for Westphal balance.

In using the Westphal balance the temperature of the solution is read from the thermometer of the sinker. If the solution is turbid or dark-colored, rendering the reading of this thermometer difficult or impossible, the temperature is read either by carefully drawing up the thermometer body until the top of the mercury column is visible, or, better, by means of a larger thermometer immersed in the solution. Thermometers and cylinders of special form have been constructed for taking specific gravities, a type of which is shown in Fig. 31.

The later models of the Westphal balance for the sugar industry are calibrated to indicate the apparent specific gravity at 20° C., so that they may be used in connection with the table of Domke. To obtain the same result with a balance calibrated for 15° C., it is only necessary to reduce the weight of the two large riders by filing off 4 mg.[37] The smaller riders need not be changed because the correction falls within the error of the instrument.

FIG. 32.
Hydrometer.

The sinker should always be immersed in the liquid to the same depth as in checking the balance with distilled water. Care must also be taken that not only the twisted portion of the wire attached to the rider is completely in the liquid, but also at least an equal length of single wire. The wire must be perfectly clean to avoid errors due to unequal wetting.

Hydrometers. A third method of determining the specific gravity of sugar solutions, and the one most commonly employed in technical operations, is by means of the hydrometer. In its usual form (Fig. 32), this instrument consists of a hollow glass body terminating at its lower extremity in a bulb (which can be weighted with mercury or shot) and at its upper extremity in a hollow slender stem, inside of which a paper scale is sealed. If this instrument is allowed to float in a solution, the weight of liquid displaced is equal to the weight of the floating hydrometer. If placed in solutions of different concentration, the stem will sink to varying depths; that point upon the scale which is level with the surface of the liquid indicates the specific gravity or percentage for the given concentration and temperature. It is in this manner that hydrometers are calibrated and standardized.

In actual practice a hydrometer scale is standardized at only a few of its points, the intermediary divisions being determined by interpolation. The method of interpolation will depend upon whether the scale is to indicate specific gravity or direct percentages.

The specific gravity D of a solution is equal to the weight W of the hydrometer divided by the volume V of the part submerged. Then $V = W/D$. If the scale is to be graduated for specific gravity the numerical divisions will proceed in arithmetical progression, such as 1.00; 1.05; 1.10; 1.15; 1.20, etc. The difference between the volumes

[37] Brendel, *Z. Ver. deut. Zucker-Ind.*, **73, 27 (1923).**

of the hydrometer for any two scale divisions will give the volume v between these divisions; letting r = half the diameter of the stem, then $v/\pi r^2$ = the distance between the two divisions. The relationship between the stem divisions of a hydrometer weighing 20 g. and with a cross area of stem (πr^2) equal to 0.2 sq. cm. can be seen from Table XXI.

TABLE XXI

SHOWING HYDROMETER SCALE DIVIDED ACCORDING TO SPECIFIC GRAVITY

Specific Gravity D	Volume of Part Submerged $\frac{20}{D}$	Volume between Divisions v	Distance between Divisions $\frac{v}{0.2}$
	ml.	ml.	cm.
1.00	20.000		
		0.952	4.76
1.05	19.048		
		0.866	4.33
1.10	18.182		
		0.791	3.96
1.15	17.391		
		0.725	3.63
1.20	16.666		
		0.666	3.33
1.25	16.000		
		0.615	3.08
1.30	15.385		

It will be noted that as the specific gravity increases the distance between the scale divisions decreases. Owing to the great labor involved in the making of calculations and measurements, the division of a hydrometer scale harmonically is accomplished in practice by means of a dividing engine.

In the graduation of a hydrometer scale for indicating direct percentages of sugar, the distance between the scale divisions is much more uniform. The relationship is best seen from the following table, where a hydrometer of 20-g. weight and 0.2 sq. cm. cross area of stem (πr^2) was used as before.

The maximum difference between the length of the scale divisions in Table XXI is 1.68 cm., while for the same range of specific gravity the maximum difference of Table XXII is only 0.26 cm. For a hydrometer graduated to read direct percentages of sugar, it is customary in practice to establish only a few points upon the scale by means of sugar solutions of known concentration, and then divide the intervals

between these points into equal subdivisions. Though this method is not absolutely accurate, the errors of division are less than the probable errors of observation.

TABLE XXII

HYDROMETER SCALE DIVIDED ACCORDING TO SUGAR PERCENTAGE

Percentage Sugar	Specific Gravity D	Volume of Part Submerged $\frac{20}{D}$	Volume between Divisions v	Distance between Divisions $\frac{v}{0.2}$
		ml.	ml.	cm.
0.00	1.00000	20.000		
			0.772	3.86
10.00	1.04014	19.228		
			0.766	3.83
20.00	1.08329	18.462		
			0.758	3.79
30.00	1.12967	17.704		
			0.747	3.74
40.00	1.17943	16.957		
			0.733	3.67
50.00	1.23278	16.224		
			0.719	3.60
60.00	1.28989	15.505		

The construction of a hydrometer to read direct percentages of sucrose is first due to Balling. The scale of this instrument, as afterwards recalculated by Brix, constitutes the form at present in most general use. The divisions of the scale are usually called degrees Balling or degrees Brix, as the case may be; the differences between the two scales are so slight that they have no significance in practical work.

The Brix hydrometer[38] or spindle is supplied in a variety of forms. For approximate work spindles are used with graduation of 0–30, 30–60, and 60–90, and divided either into 0.5° or 0.2°. The forms in most common use, however, have only a range of 10°, 0–10, 10–20, 20–30, 30–40, etc., graduated into 0.1°. For greater accuracy a third form of spindle has been made with a range of only 5°, 0–5, 5–10, 10–15, 15–20, etc., and graduated into 0.05°. With the help of a spindle for only approximate work, the choice of the particular hydrometer for the finer reading will be facilitated. The accuracy of the spindle is of course the greater, the smaller the diameter of the stem and the consequently larger interval between the scale divisions.

[38] The term saccharometer, which is sometimes applied to a hydrometer indicating percentages of sucrose, is unfortunate, owing to the confusion with the word saccharimeter, of entirely different meaning.

In determining specific gravity by means of the hydrometer, a tall, narrow cylinder is usually employed for holding the liquid to be examined. The spindle is carefully lowered into the solution in such a way that the surface of the stem above the liquid is not moistened. The best method is to sink the hydrometer very slightly below the point where it floats naturally and then release it.

Care should also be exercised that the instrument floats freely and does not touch the bottom or walls of the cylinder. The reading is made by bringing the eye upon a level with the surface of the solution

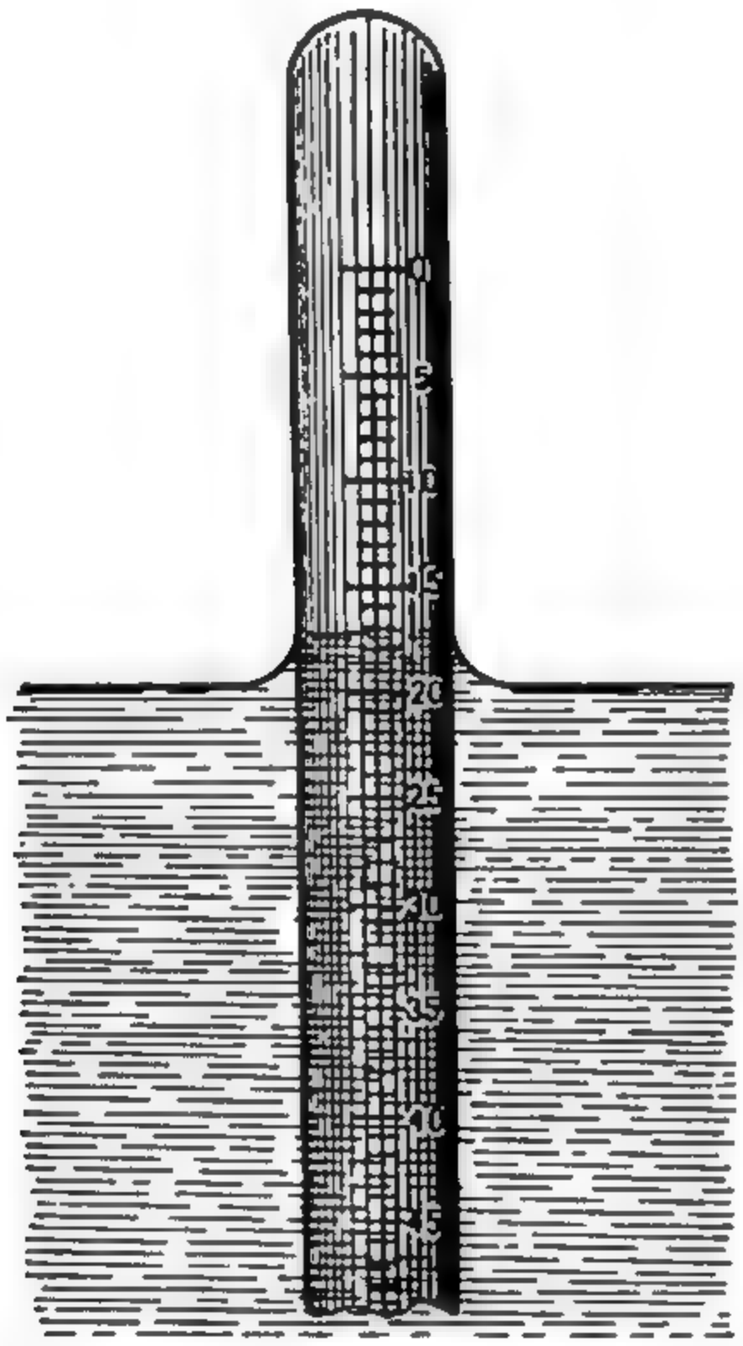


Fig. 33. Floating Brix spindle.

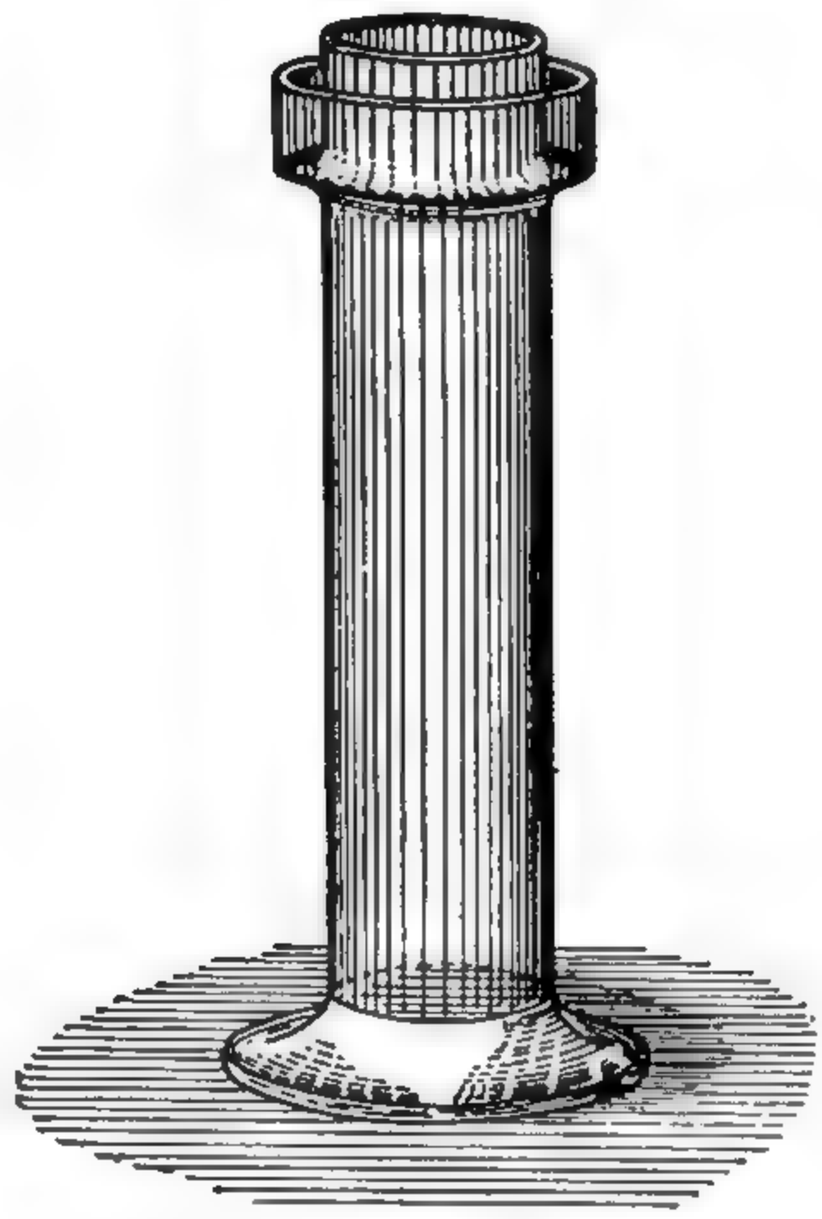


Fig. 34. Winter's cylinder for taking specific gravity.

and noting where the border line intersects the scale; the film of liquid drawn up around the stem by capillarity should be disregarded. The reading of the spindle, for example, in Fig. 33, is 20 and not 17. The scale of the hydrometer is read with greater ease when the surface of the liquid is level with the brim of the cylinder. Cylinders of the form designed by Winter (Fig. 34) are convenient for this purpose; any overflow of liquid displaced by the spindle is caught in the circular trough.

The same attention must be paid to temperature when the hydrometer is employed as in other methods of determining specific gravity. The Brix determination of dark liquids is facilitated by illuminating the

surface of the liquid from below with a light held behind the cylinder. This gives a sharp line of separation at the surface of the liquid.

The original Brix spindle was calibrated at 17.5° C., and some of these old-style hydrometers are still in use. The modern new-style Brix hydrometers are calibrated at 20° C.; for tropical countries spindles have also been calibrated for use at 25° C., 27.5° C., and 28° C. Unless sugar solutions have the same temperature as that at which the hydrometers are calibrated a correction must be applied to the readings. For the readings of the new-style Brix hydrometers of 20° C. calibration, the corrections of Table 2 of the Appendix should be applied; the same table may also be used to correct readings obtained on the original (17.5° C.) scale, as explained in the text below the table.

Brix hydrometers are sometimes fitted with thermometers, a form of which modification is shown in Fig. 35. The advantages of this construction disappear somewhat when working with turbid liquors, which render the reading of the thermometer difficult or impossible. For general purposes the temperature of the solution is best taken by means of an accurately standardized special thermometer.

Volquartz[39] has constructed a Brix spindle with a correction scale, the mercury of the thermometer in the stem indicating, instead of temperature, the correction necessary to be added to the scale reading. The method of operation may be seen from Fig. 36. The spindle in the illustration indicates 10.0 Brix; the mercury of the thermometer marks 2.7; the corrected reading is, then, 10.0 + 2.7 = 12.7 Brix. If the mercury is below the 0 mark, the correction must be subtracted.

Horne has devised a hydrometer (Fig. 37) with a double correction scale, reading tenths of degrees Brix and easily estimated to hundredths. The two scales, one applicable to the higher and the other to the lower readings of the spindle, lie on either side of the thermometer, the mercury of which indicates the correction. Temperature corrections for intermediate readings of the spindle are readily interpolated.

Deerr's Brixometer. In order to increase the accuracy of reading the Brix hydrometer, Deerr[40] has designed a special form of apparatus, known as the Brixometer or dasymeter. It was originally constructed of metal and was somewhat complicated. The improved model, Fig. 38, is made of glass. The cylinder *A*, in which the hydrometer floats, is mounted on a foot which is ground horizontal. A saucer, *E*, provided with an outflow nipple at the rim, is cemented to the cylinder

[39] *Z. Ver. deut. Zucker-Ind.*, 46, 392 (1896).

[40] *Intern. Sugar J.*, 23, 208 (1921); 35, 476 (1933). The apparatus is manufactured by Baird and Tatlock, 14 Cross Street, Halton Garden, London, E.C.1.

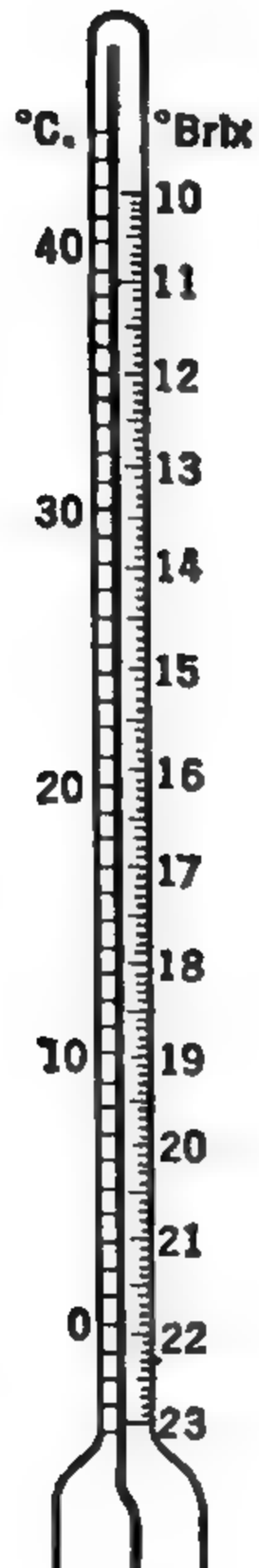


Fig. 35. Brix spindle with thermometer.

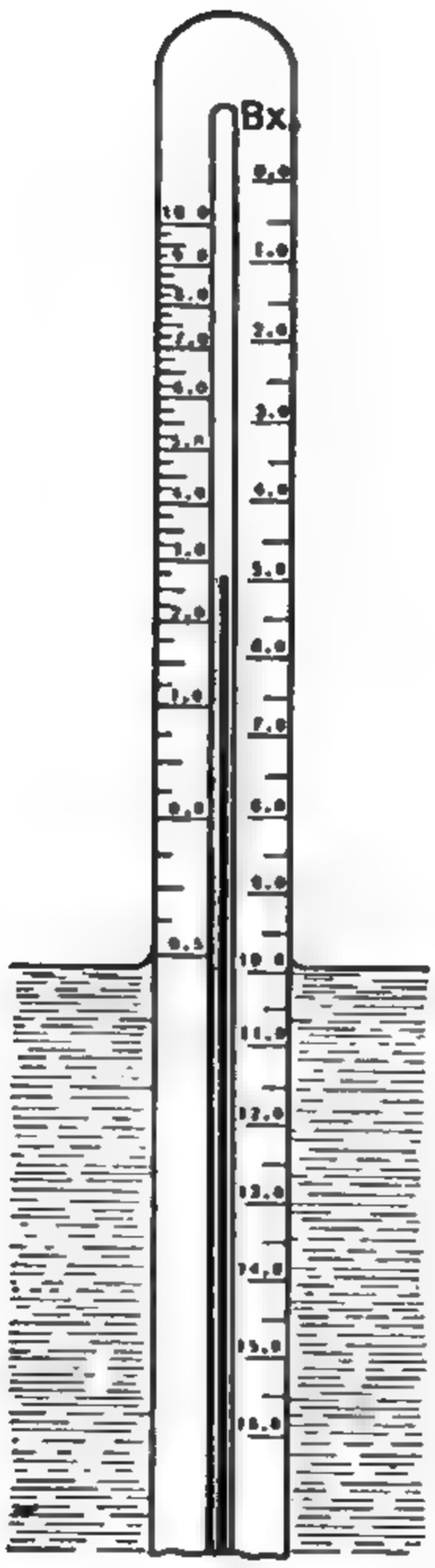
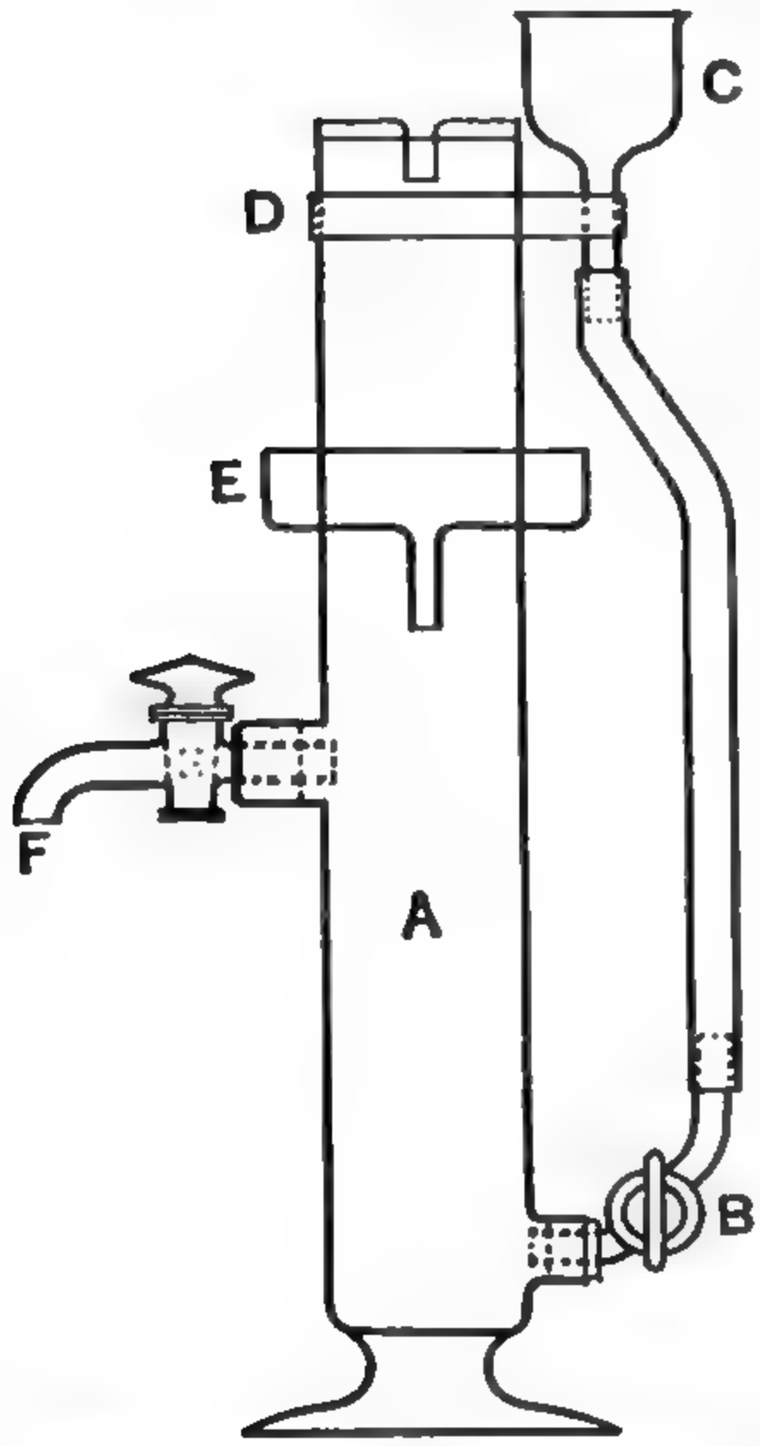


Fig. 36. Volquartz spindle with temperature correction scale.

Fig. 37. Horne's Brix spindle with complete temperature correction scale.

to catch the overflow, as in Winter's Brix cylinder (p. 75). At the lower end of the cylinder is a side tube with stopcock *B*, which is connected by rubber tubing with the reservoir *C*, held by spring clip *D*. The unique feature of Deerr's device is that the hydrometer is read not at the surface of the liquid, where there is always some uncertainty as to the proper index level, but at a fixed point above the surface. A rectangular slot, 1.5 cm. high by 0.75 cm. wide, is cut out of the upper end of the cylinder, producing a constant level of the liquid in the cylinder. One centimeter above the level of the lower edge of the slot a fine horizontal line is engraved around the cylinder, serving as the reference point.



(Reproduced with permission from "Methods of Control, Sugar Technologists' Association of India," p. 81, 1936.)

FIG. 38. Deerr's Brixometer.

The cylinder is filled through reservoir *C*, with stopcock *B* open, and the hydrometer inserted. The stopcock is closed and a little more of the solution poured into the reservoir. On opening the stopcock again the liquid overflows through the slot and forms a constant level. The eye of the observer is leveled so that the reference mark around the cylinder appears as a uniform straight line; the point where this line appears to cut the hydrometer scale is taken as the reading. By this means readings to 0.01° Brix may be made, but such an accuracy is obtained only if the temperature of the liquid is determined within 0.1° C. The Brix spindle must also be accurately standardized. Deerr recommends spindles with a range of 8° Brix each, and with 2-mm. distance for each 0.1° Brix. The correction to be applied to the reading for the distance between the surface of the liquid and the reference mark must be determined for each cylinder and each spindle, by means of solutions the Brix of which has been accurately determined by the pycnometer method. The stopcock *F* at the side of the cylinder is used to withdraw liquid for other determinations, as polarization, etc., to be made on the same sample.

Brix Determination in Molasses by "Double Dilution." Owing to the difficulties encountered in determining the specific gravity of molasses and other highly viscous products in the original state with the pycnometer or spindle, it is a common practice in the molasses trade to dilute the sample with an equal weight of water, take the Brix of the

solution by hydrometer, and multiply the result by 2. The solution must be allowed to stand a sufficient time for the air bubbles to rise and the suspended matter to settle out.

If a solution of pure sucrose is diluted in this manner, the Brix reading is exactly one-half of that of the original because the contraction upon dilution is already considered in the graduation of the Brix spindle and in the specific-gravity tables. But with molasses a higher result is always obtained because the contraction is greater than in pure sucrose solutions of the same original density. The magnitude of the additional contraction varies with the original density and with the nature and quantity of the non-sugars.

The extent of the excess contraction may be seen from Table XXIII, which gives figures obtained by Paar[41] for beet molasses, and those calculated from observations by Snyder[42] on Cuban blackstraps.

TABLE XXIII

CONTRACTIONS OF BEET AND CANE MOLASSES ON DILUTION WITH AN EQUAL WEIGHT OF WATER

	Brix Original	Brix 1 : 1	Contraction for 1 Kg. Molasses Solution	Contraction for 1 Kg. Sucrose Solution	Excess Contraction Molasses Solution
Beet molasses			ml.	ml.	ml.
1	80.7	82.4	8.2	5.2	3.0
2	74.9	76.2	6.6	4.2	2.4
3	79.3	80.4	7.0	5.0	2.0
4	79.15	80.2	6.9	5.0	1.9
5	78.55	80.1	7.7	4.8	2.9
6	77.2	78.4	6.8	4.6	2.2
7	76.3	77.5	6.7	4.8	1.9
Average	78.0	79.3	7.1	4.7	2.4
Cane molasses					
1	85.61	87.84	10.3	6.2	4.1
2	86.20	88.24	10.0	6.3	3.7
3	87.51	89.38	10.1	6.6	3.5
4	85.65	87.16	8.9	6.2	2.7
5	85.14	86.96	9.4	6.1	3.3
6	87.17	89.04	10.0	6.6	3.4
7	85.70	87.68	9.8	6.2	3.6
8	85.33	87.32	9.8	6.2	3.6
9	85.31	87.32	9.8	6.2	3.6
10	84.97	87.66	11.0	6.0	5.0
Average	85.86	87.86	10.0	6.3	3.7

The average correction to be applied to the Brix found by double dilution is —1.3 for normal beet molasses, and —2.0 for Cuban cane blackstraps.

[41] *Deut. Zuckerind.*, 60, 997 (1935).

[42] *J. Assoc. Official Agr. Chem.*, 15, 194 (1932).

Sweet-Water Spindles. For determining the Brix of dilute sugar solutions, an operation of considerable importance in washing filter-press cake or decolorizing charcoal (" sweetening off "), a type of hydrometer, known as the " sweet-water " spindle, has been constructed. This hydrometer has a large body with a thin stem, so that the readings can be easily made to 0.1°. The sweet water as it comes from the filters usually has a temperature of 60° to 80° C., and, to prevent the delay incident to cooling the solution to 20° C., sweet-water spindles are often calibrated at high temperatures. One form of such spindle is graduated to read 0° Brix in water at 75° C., and 5° Brix in a 5 per cent sugar solution of the same temperature; such a spindle, of course, cannot be employed at other temperatures, so that its usefulness is somewhat limited.

Fig. 39. Sweet-water spindle.

Another form of sweet-water spindle (Fig. 39) is graduated from 0° to 5° Brix in the normal way. Above the 0 mark the divisions are continued in the same manner, the result being a double scale with the 0 division in the middle. At 20° C. the readings of the lower scale give the true Brix; at temperatures above 20° C., sweet waters will read less than the true Brix. At 70° C. a 5 per cent sugar solution reads 0 on the spindle, a 4 per cent solution —1, a 3 per cent solution —2, a 2 per cent solution —3, a 1 per cent solution —4, and pure water —5. The true Brix can be determined for any temperature by means of a correction table; determinations by this instrument can always be controlled by cooling the solution to 20° C.

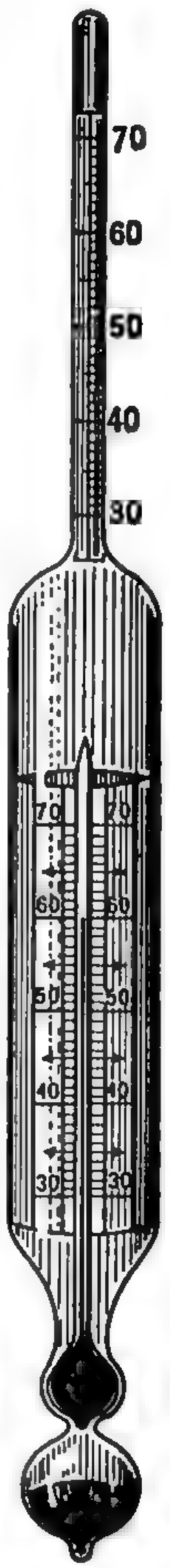


Fig. 40. Langen's sweet-water spindle.

Still another form of sweet-water spindle has been devised by Langen. This spindle (Fig. 40) contains within its body a thermometer graduated from 30° to 70° C. The graduated scale in the stem of Langen's spindle differs from other forms, however, in not giving Brix degrees, but in simply indicating the thermometer reading for each division to which the

hydrometer will sink in pure water. If placed, for example, in distilled water of 30° C., the instrument will sink to the division 30 on the stem, and in water of 70° C. to the division 70; in other words, the thermometer and scale of the spindle will give the same readings between 30 and 70 when the instrument is floated in distilled water. When the spindle is placed in a sweet water, the reading of thermometer and scale will no longer agree. The spindle necessarily sinks to a lesser depth than in water, and the scale of the stem gives a different reading from that of the thermometer, the difference between the two being proportional to the concentration of the solution. In sweetening off, it is only necessary to observe the readings of thermometer and scale; the differences between these decrease as the extraction proceeds, until with the coincidence of the two readings complete exhaustion is indicated.

Baumé Hydrometers. Another form of hydrometer which is frequently used in the sugar factory, but to a much less extent in the sugar laboratory, is that of Baumé. This instrument is standardized by means of common salt; the 0 point at the top of the stem is obtained by means of distilled water, and the 15° mark by means of a 15 per cent salt solution. The interval between these two divisions is then divided into 15 equal parts, this graduation being extended downwards on the scale as far as desired. Unfortunately, in the early instruments the temperature of the water and the specific gravity of the salt solution were not correctly obtained, so that the values of the Baumé scale divisions have been variously reported by different authorities. The so-called "old" Baumé degrees, as calculated by Brix, are still used in some countries in the commercial analysis of molasses notwithstanding the fact that Gerlach as long ago as 1870 showed the incorrectness of the formulas employed by Brix in his calculations.

Gerlach found 1.11383 as the specific gravity of a 15 per cent salt solution at 17.5° C. The volume of a Baumé spindle up to the 0 mark, in terms of the volume of a single scale division, is then equal to $\frac{1.11383 \times 15}{1.11383 - 1} = 146.78$. The specific gravity S corresponding to any scale division N of the Baumé scale can then be calculated by the formula $S = \frac{146.78}{146.78 - N}$. It is by use of this formula that the so-called "new" Baumé degrees have been determined. The constant used to convert specific gravity into degrees Baumé (146.78 for the "new" scale) is termed the "modulus." Its value for the "old" scale is 144.

The U. S. Bureau of Standards[43] has introduced a more recent Baumé

[43] *Tech. Paper Bur. Standards* 115, 1918.

scale for sugar solutions, the tables of which are calculated according to the formula:

$$\text{Degrees Baumé} = 145 - \frac{145}{\text{True Specific Gravity at } \frac{20^\circ}{20^\circ}\text{ C.}}$$

The Baumé degrees thus found (modulus 145) lie between the so-called "old" and "new" degrees, being in almost perfect agreement with the old up to 25 per cent sugar, and above this point about 0.1° to 0.2° higher than the old. The Baumé scale of the Bureau of Standards has been quite generally accepted by the molasses trade in the United States.

The relationship between percentage of sugar or degrees Brix, apparent specific gravity at $\frac{20^\circ}{20^\circ}$ C., degrees Baumé according to the Bureau of Standards scale, and weight per gallon at 20° C. is shown in Table 3 in the Appendix. Temperature corrections for Baumé hydrometers calibrated according to the Bureau of Standards scale are given in Table 4, and those for the weight per gallon in Table 5.

For molasses and other viscous solutions Baumé degrees are sometimes taken at 100° F. For this purpose[44] the vessel of molasses is immersed in boiling water for an hour to remove air bubbles. A glass cylinder is then filled with the molasses, care being taken to remove any foam, and a thermometer is inserted. When the temperature has fallen almost to 100° F. (38° C.), the hydrometer is placed in the molasses and the reading taken at exactly 100° F.

Still another method for determining degrees Baumé is used in the corn products industry. The cylinder containing the commercial glucose sirup is placed in a water bath heated to approximately 140° F., and the Baumé spindle is introduced. The scale reading and the exact temperature are taken, and then the scale reading is corrected to 100° F. by adding 0.1° Baumé for every 4° F. The "old" Baumé scale has been generally used in the corn products industry for many years, but it is gradually being replaced by the Bureau of Standards scale.

The Baumé scale, owing to its conventionality and the confusion in standards, should be abandoned[45] in sugar analysis for the more rational Brix scale.

Relationship between Brix and Dry Substance. Because of the equipment and time required for dry substance determinations, numerous methods have been proposed for converting Brix readings into the actual percentage of dry substance. They are usually based on the

[44] Great Western Sugar Co., "Methods of Analysis," p. 101, 1920.

[45] The Baumé scale was officially discontinued in France in 1919 and is no longer employed in the public service for determining the density of sugar and other carbohydrate solutions.

fact that the salts present in most sugar products increase the specific gravity over that which a sucrose solution of the same concentration would have. All such calculations give only approximate results because the proportions between the various salts show large variations.

According to Sijlmans[46] the dry substance in Java molasses can be calculated by multiplying the sulfated ash (uncorrected) by a factor and subtracting the product from the Brix of the molasses, obtained by diluting 1 part of molasses with 9 parts of water by weight and multiplying the Brix found by 10. The average ash factor for defecation molasses is 0.72, for sulfitation molasses 0.86, and for carbonatation molasses 0.74. Haddon[47] gives for Natal molasses the factor 0.875, by which the carbonated ash must be multiplied to calculate the correction to the Brix, found by diluting 100 g. molasses with water to 500 ml.

King[48] found by the analysis of a large number of Philippine molasses samples that the logarithm of the carbonated ash is a straight-line function of the difference between the dry substance and the Brix determined by diluting the molasses to the approximate density of mill juice. Fort[49] showed that the dry substance in Louisiana raw juices can be determined approximately by multiplying the Brix by the carbonated ash per 100° Brix, multiplying the product by the average factor 0.0120, and subtracting the result from the observed Brix. For evaporator sirup, diluted to juice density, the factor is 0.0108 instead of 0.0120.

King observed that the Brix of the molasses samples examined by him was an approximately straight-line function of the dry substance, and Davies[50] was able, by statistical analysis, to establish equations for the relationship between the two.

All such methods must be used with great caution because the relationship varies with location, variety of cane or beet, agricultural practices, and other factors. They are useful, however, to detect abnormalities in the composition of sugar products.

Commercial glucose sirup and crude dextrose contain so little mineral matter that it has no practical effect on the ratio between the dry substance and the solids based on density. This ratio varies, however, with the " purity " or " dextrose equivalent " of the product, that is the percentage of reducing sugars, expressed as dextrose, in the total solids. Fetzer and Evans[51] have found that the dry substance corresponding to a given degree Baumé increases with the purity. The Brix reading for

[46] *Arch. Suikerind.*, 40, II, 993 (1932).
[47] *Rev. agr. Maurice* No. 66, 229 (1932).
[48] *Ind. Eng. Chem., Anal. Ed.*, 3, 230 (1931).
[49] *Ind. Eng. Chem., Anal. Ed.*, 8, 333 (1936).
[50] *Trop. Agr.*, 9, 218 (1932).
[51] *Ind. Eng. Chem., Anal. Ed.*, 7, 41 (1935)

a commercial glucose sirup is always greater than the dry substance. When such a sirup is diluted with water the dry substance decreases more rapidly than the Brix reading. For crude dextrose in dilute solution the Brix reading is also higher than the dry substance, but for concentrated solutions it is lower. Fetzer and Evans have calculated a table, giving the actual moisture content of sirups of varying degree Baumé and for purities ranging from 40 to 92, on either a dry-substance or Brix basis. Later they supplemented this table, applicable only to heavy sirups, by another one,[52] for solutions of sirups or corn sugar, ranging from 10° to 70° Brix. This table shows the factors by which the Brix must be multiplied to obtain the dry substance, for varying purities on the Brix basis, and also the corrections that must be applied to convert Brix purities into dry substance purities.

[52] *Ind. Eng. Chem.*, **28**, 885 (1936).

CHAPTER IV

PRINCIPLE AND USES OF REFRACTOMETERS

A second method of estimating the percentage of sugars in solution is by means of the refractive index. The general applicability of this method, as in the case of specific gravity, depends upon the fact that solutions of all sugars of equal concentration have nearly the same index of refraction.

Law of Refraction. If a beam of light from one medium, such as air, falls at an inclined angle upon the surface of a second medium, such as water, it will be found that the beam upon entering the second medium is bent or deflected from its original course. A good example of this phenomenon, which is called refraction, is the bent appearance of the oar of a boat when seen obliquely under water. There is a general law of refraction for all transparent liquids and solids which may be stated as follows: For two given media and the same ray of light (same wavelength), the ratio of the sine of the angle of incidence to the sine of the angle of refraction is always a constant quantity for the same temperature.

In Fig. 41 m and m' are two media; PP' is drawn perpendicular to the dividing surface FF'. Let a beam of light pass through m in the direction LO; a part of the beam at the point O of the surface is reflected in the direction OL'; another part of the beam entering m' is refracted in the direction OS. The angle LOP which the falling ray makes with the perpendicular is the angle of incidence, or i; the angle SOP' which the refracted ray makes with the perpendicular is the angle of refraction, or r. The ratio $\frac{\sin i}{\sin r} = n$ is called the index of refraction. This ratio in Fig. 41 is represented by $\frac{\text{line } ab}{\text{line } cd}$.

The ratio $\frac{\sin i}{\sin r}$ is also that of the velocities of light in the two media. If v is the velocity of light in m and v' the velocity in m', then $n = \frac{\sin i}{\sin r} = \frac{v}{v'}$. If the refracted ray is bent toward the perpendicular as in Fig. 41, the velocity v' is smaller than v, and the medium m' is called of greater optical density than m. Optical density must not be

confused with material density, since the two expressions do not at all correspond.

If the ray of light in Fig. 41 passes from a denser medium m' into a rarer medium m in the direction SO, it will be refracted in m in the direction OL. Then the index of refraction is $\frac{\sin r}{\sin i}$, which is the

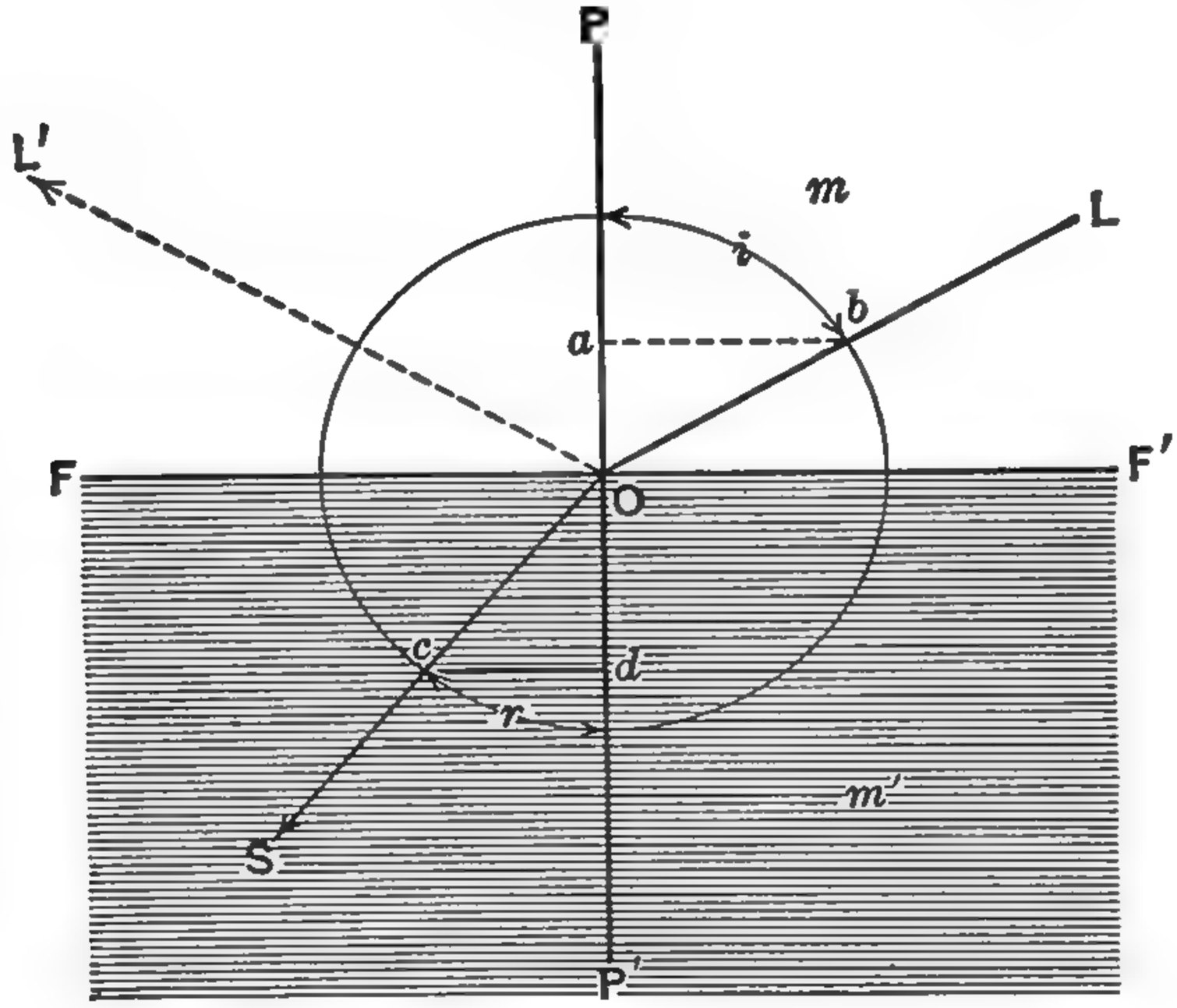


FIG. 41. Illustrating law of refraction.

reciprocal of the index for light passing in the opposite direction. The refractive index varies with the wavelength of the light, increasing from the red towards the violet end of the spectrum. From this it follows that when ordinary light is refracted it is decomposed into light of the different prismatic colors; this unequal refraction for light of different wavelengths is called dispersion.

Measurement of Refractive Index. The refractive index of a solution can be measured in a variety of ways. One of the simplest methods, which is of more value for demonstration than for accuracy, is by means of the refractometer trough. This apparatus, shown in Fig. 42, consists of a semicircular trough, the inner curved surface of which is divided into degrees. The side of the trough corresponding to the diameter of the circle consists of a plate of glass which is made non-transparent, except a narrow perpendicular slit at the center *c*. If the trough is filled partly with a solution and a beam of light falls upon the glass, that part of the beam passing through the slit above the surface of the liquid will mark the angle of incidence and that part passing

below the surface will mark the angle of refraction. In the illustration, where water is used, these angles are 60° and 40° respectively.

$$\frac{\sin 60°}{\sin 40°} = \frac{0.8660}{0.6428} = 1.34 \text{ or } n, \text{ the approximate index of refraction}$$

In the construction of refractometers for more accurate measurements, instrument makers generally employ the method of total reflection. The principle of this method can be understood from Fig. 43.

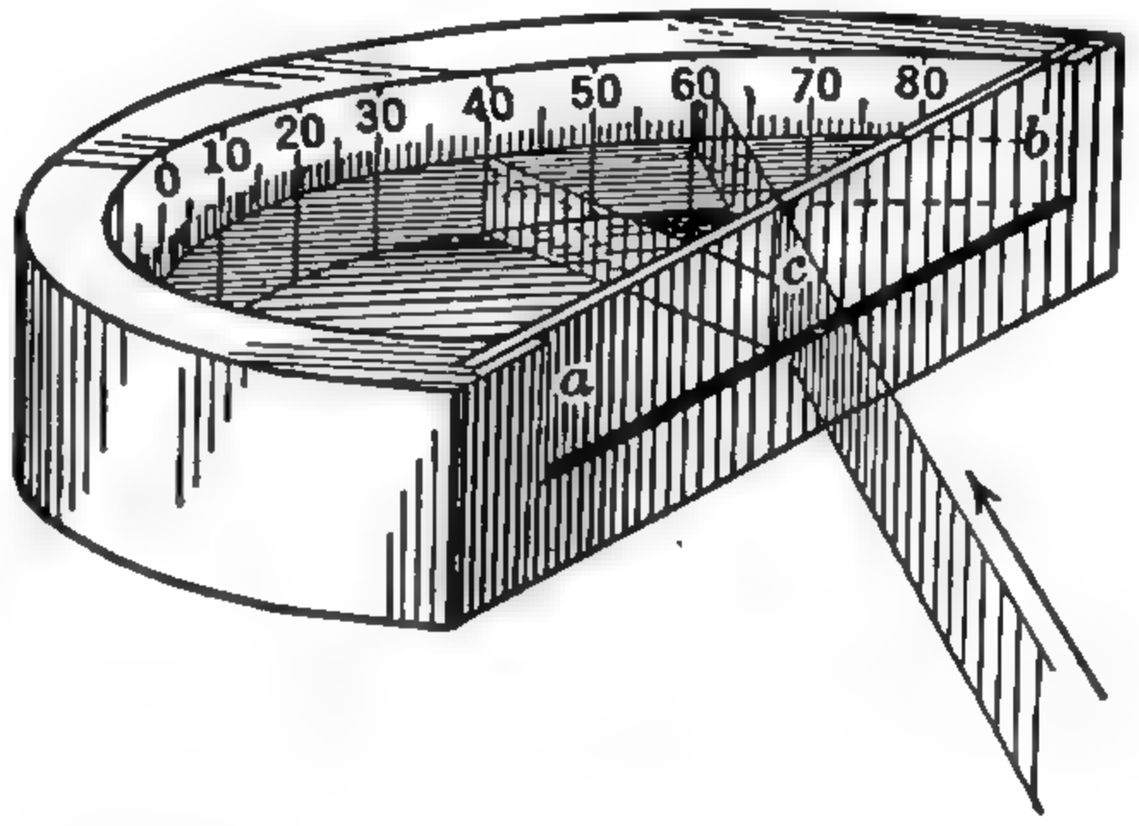


FIG. 42. Measuring refractive index by refractometer trough.

Let *m and* m_1 be two media, such as glass and water, of which *m* is the more optically dense, the dividing surface being *SF*. The beams of light which fall from the source *L* upon *SF* at various angles are refracted, in m_1 in different directions. The beam $LO \perp SF$ is not refracted and proceeds in the same direction; the beam *Lo*, making the angle of incidence *i*, is refracted in the direction *ot*, making the angle of

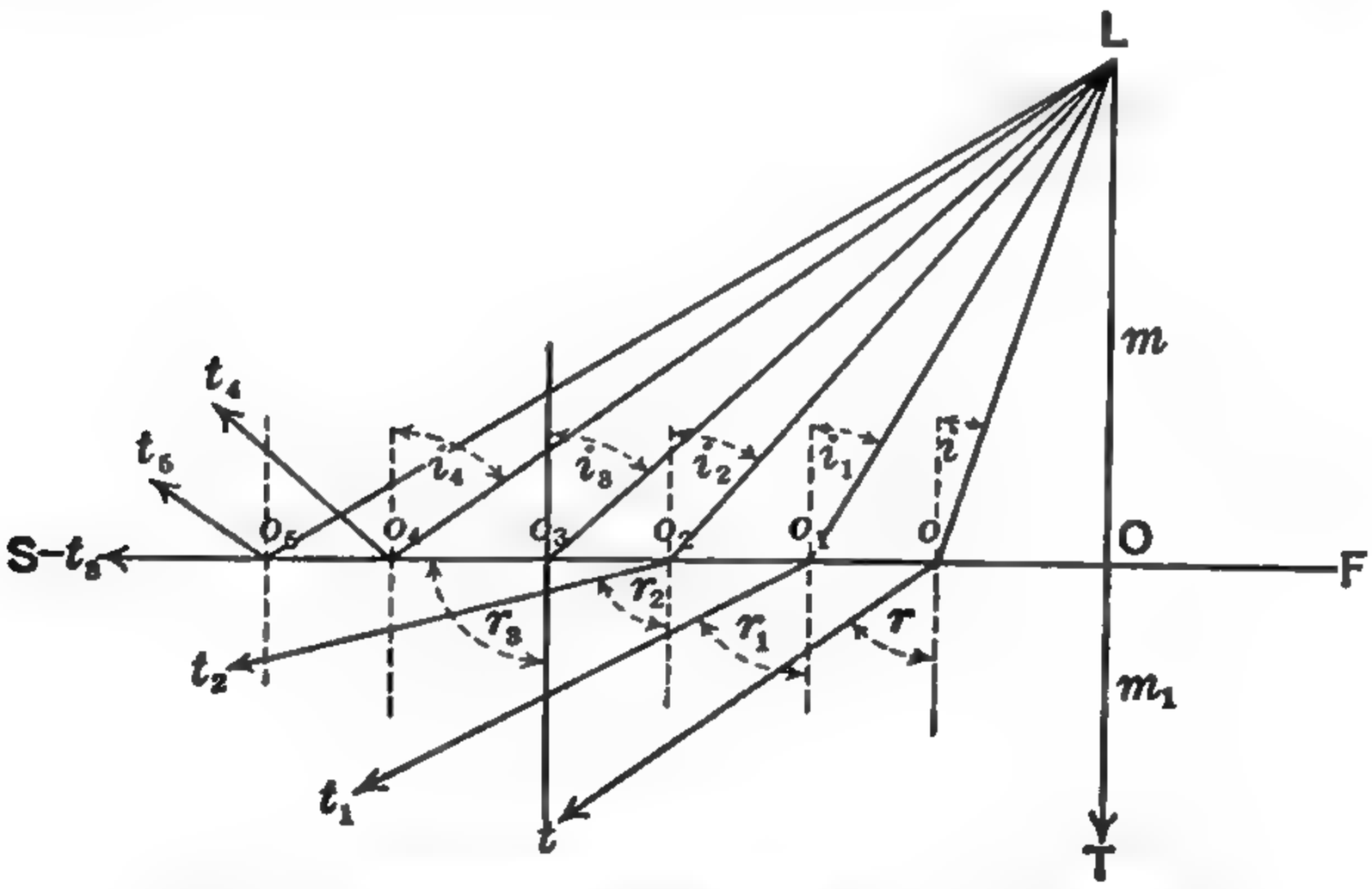


FIG. 43. Illustrating principle of total reflection.

refraction *r*; in the same way Lo_1 is refracted to o_1t_1, and Lo_2 to o_2t_2. As the angle of incidence for the falling beam increases, there finally comes a point at o_3 where the refracted ray o_3t_3 coincides with the surface *SF*, and the angle of refraction $r_3 = 90°$. If the angle of in-

cidence is increased beyond i_3 to i_4, the beam which previously was only partly reflected is totally reflected in the direction t_4, and there is no refraction in m_1. Since $\frac{\sin i_3}{\sin r_3}$, the index for the beam before total reflection, equals $\frac{\sin i_2}{\sin r_2}$, etc., $= \frac{\sin i}{\sin r} = n$, and since $\sin r_3 = 90° = 1$, it is evident that for the borderline of total reflection $\sin i = n$. In other words, the sine of the angle of incidence for the borderline of total reflection is equal to the refractive index. It is seen from the diagram that total reflection can take place only when light passes into an optically rarer medium.

For absolute measurements the refractive index of a substance is referred to a vacuum. Since, however, the absolute index of air is only 1.000294, refractive indices referred to air are sufficiently exact for most purposes. With three media such as air, glass, and a liquid, if the index from air to glass is N_{ag} and from glass to liquid N_{gl}, then the index from air to liquid $N_{al} = N_{ag} \times N_{gl}$. The sine of the angle of incidence for the borderline of total reflection between glass and a given liquid, multiplied by the index of refraction between air and glass, will give the index of refraction for the liquid with reference to air.

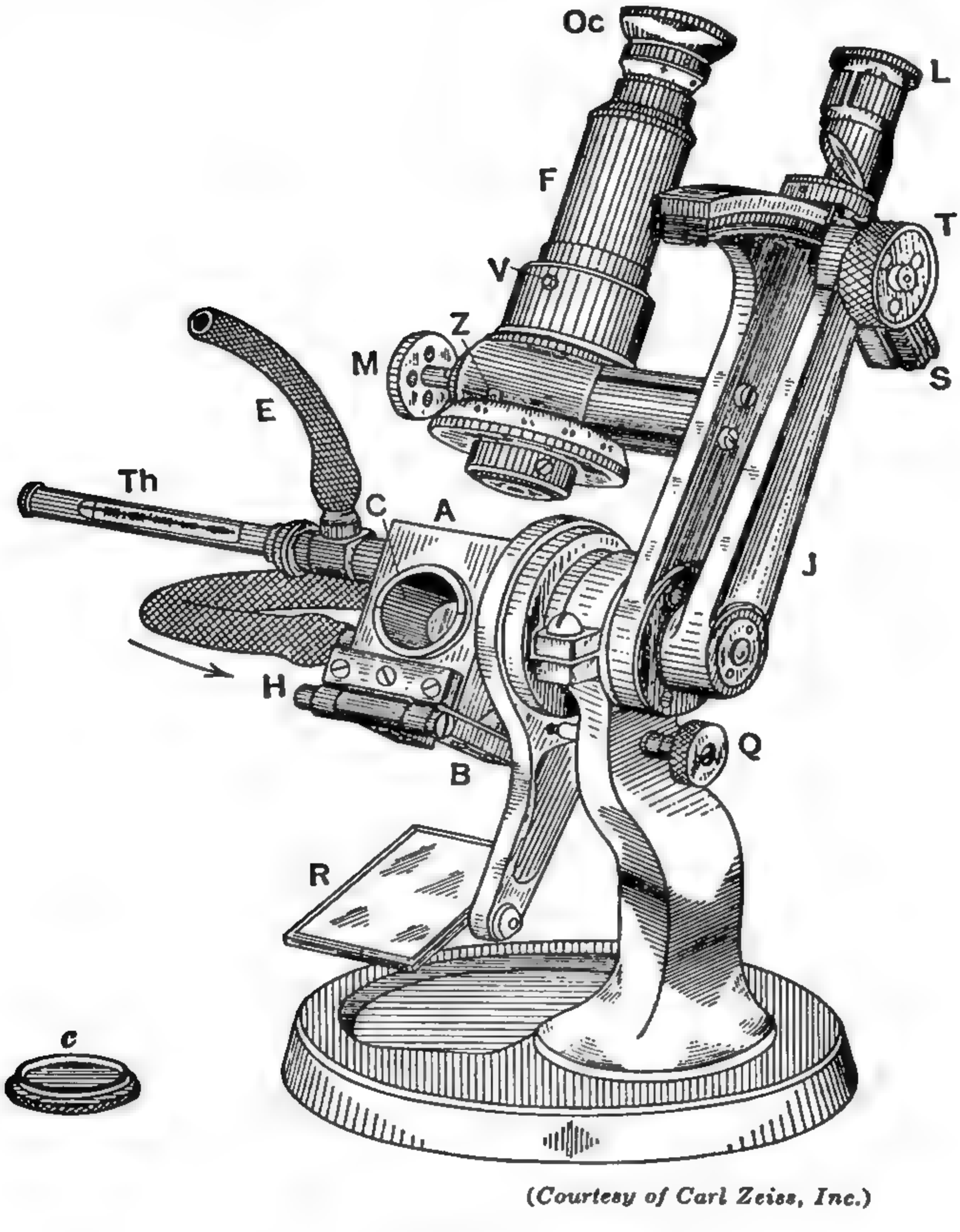
Abbe Refractometer

The best known general instrument for determining the refractive index of sugar solutions is that of Abbe (Fig. 44).[1] The essential part of the Abbe refractometer consists of two flint-glass prisms A and B of refractive index $n_D = 1.75$, each cemented into a metal mounting. To open the prisms the latter are rotated on their bearings to a horizontal position with the prism B uppermost; the clamp which holds the prisms together (not visible in Fig. 44, but shown in Fig. 47) is then released and prism B swung open on its hinge H. A few drops of the solution to be examined are then placed upon the polished inner surface of the fixed prism A next to the telescope, and prism B, whose inner surface is ground, is brought slowly back and clamped as before. The instrument is then swung into an upright position and light reflected from the mirror R upon the surface of the lower prism.

In the diagram shown in Fig. 45, FDE and ABC are longitudinal sections of the two prisms in an Abbe refractometer between whose hypotenuse surfaces FE and AB (separated by about 1.5 mm.) is the film of liquid to be examined. The beams of light passing from L

[1] Manufactured by Bausch & Lomb Optical Co., Rochester, N. Y.; Industro-Scientific Co., Philadelphia, Pa.; Spencer Lens Co., Buffalo, N. Y.; Carl Zeiss, Jena.

through the lower prism to the surface of the solution AB are refracted or totally reflected, according to the refractive index of the liquid. As shown in the diagram the beams which fall upon the hypotenuse surface AB at a less inclination than the line IO undergo re-



(Courtesy of Carl Zeiss, Inc.)

FIG. 44. Abbe refractometer.

fraction in the liquid, and, passing through the upper prism, the sets of parallel rays s, s', s'', . . . , u, u', u'', . . . , etc., are condensed by the objective K of the telescope upon the field XY. The beams in the prism parallel to IO are refracted along the surface BA and the beams of greater inclination totally reflected; since these beams do not reach the surface of the upper prism, a part of the field XY remains in shadow.

The telescope of the refractometer (F in Fig. 44) is attached to a sector S and the prisms to an arm J (the alidade) which carries a magnifying lens L and is rotated by means of a rack-and-pinion movement operated by screw head T. By moving the alidade until the intersection of the reticule in the telescope field (Fig. 45) cuts the dividing

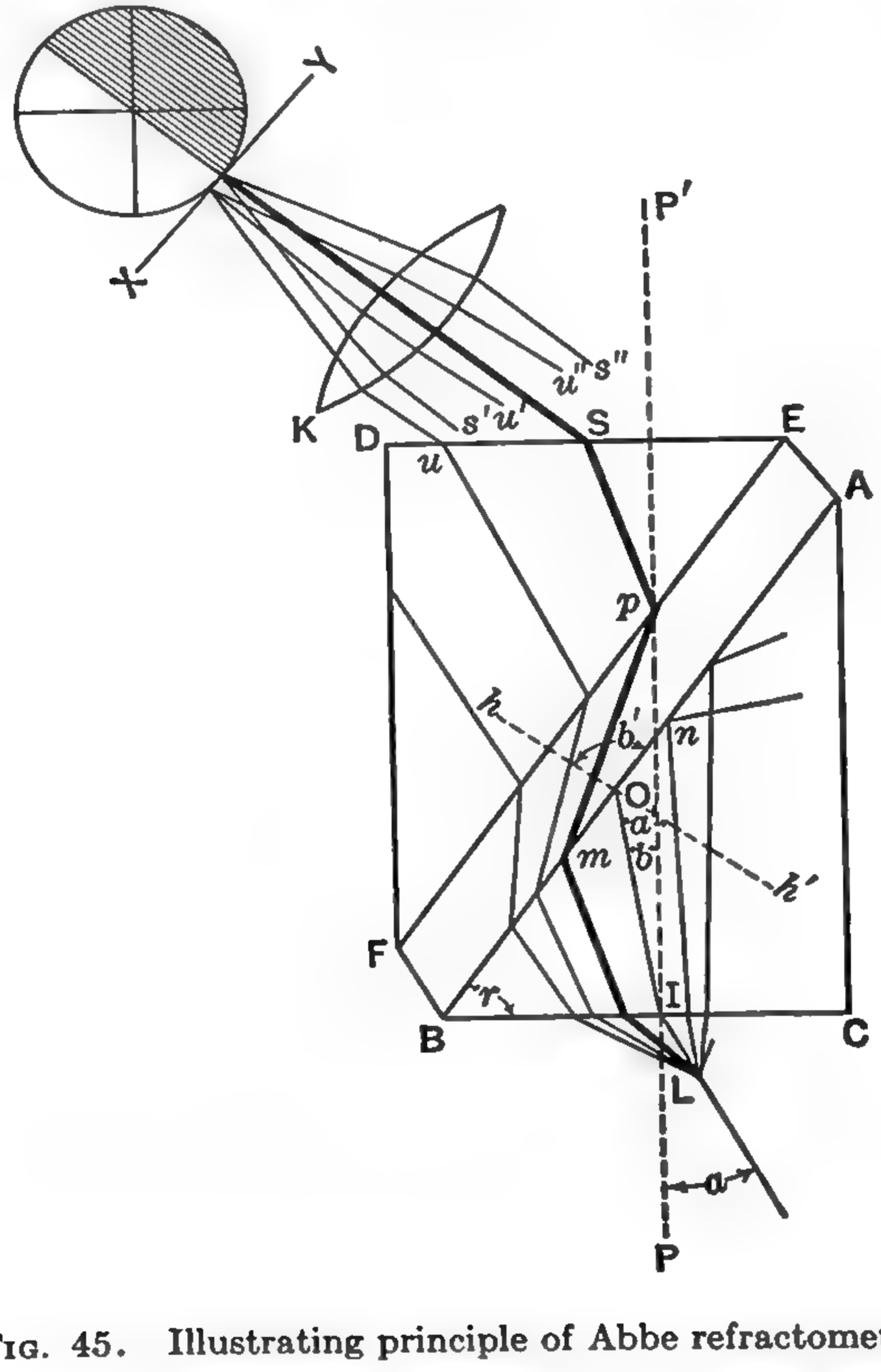


FIG. 45. Illustrating principle of Abbe refractometer.

line between the bright and dark portions of the field, the refractive index can be read directly upon the scale of the sector by means of the lens.

The relation between the angles of incidence and refraction of light between air and prism, and prism and liquid, in the Abbe refractometer may be understood from Fig. 45. Let PP' be drawn $\perp$ to the end planes BC and DE of the double prism, and hh' be drawn $\perp$ to the hypotenuse planes AB and EF.

Let a = angle of incidence from air and b = angle of refraction in glass; then

$\frac{\sin a}{\sin b} = n$ for prism, which for the flint glass of the Abbe instrument is about 1.75

Let r = angle of prism; a' = angle of incidence in glass upon surface AB; and b' = angle of refraction in liquid = 90° for borderline of total reflection.

In $\triangle BOI$,

$$\angle r + \angle BOI + \angle BIO = 2\,rt.\ \angle\text{'s}$$
$$\angle BOI + \angle a' + \angle BIO + \angle b = 2\,rt.\ \angle\text{'s}$$

whence $r = a' + b$.

By way of illustration the following values are given for a, b, and r, with water as the liquid between the prisms:

$$a = 18°\ 32'$$
$$b = 10°\ 28'$$
$$r = 60°\ 00'$$
$$\frac{\sin a}{\sin b} = \frac{0.3179}{0.1817} = 1.75 = n \text{ for air to prism}$$
$$a' = 60° - 10°\ 28' = 49°\ 32'$$
$$\frac{\sin a'}{\sin b'} = \frac{0.76}{1} = 0.76 = n \text{ for glass of prism to water}$$
$$1.75 \times 0.76 = 1.33 = n \text{ for air to water}$$

Each division, therefore, upon the sector of the refractometer representing refractive index is equal to the sine of the angle of incidence in the prism for the borderline of total reflection multiplied by the refractive index of the prism. Since total reflection can take place only when light passes from an optically denser to a rarer medium, the capacity of the refractometer is necessarily limited to solutions of smaller refractive index than 1.75.

A second important feature of the Abbe refractometer is the compensator. The function of this is to correct the dispersion which white light undergoes in the double prism. Without the compensator the borderline between the light and dark parts of the field, owing to the unequal refraction of light of different wavelengths, assumes the appearance of a band of prismatic colors, which it is impossible to use for purposes of adjustment.

The compensator of the refractometer is placed in the prolongation of the telescope tube between the objective and the double prism. It

consists of two similar Amici prisms, such as are used in a direct-vision spectroscope, and which give no divergence for the yellow D line of the spectrum (i.e., the emergent D rays are parallel with the optical axis). The two prisms are rotated simultaneously in opposite directions by means of the screw head *M* (Fig. 44).

Trapezoidal sections of the two Amici prisms are shown in Fig. 46. Each prism consists of a combination of two crown-glass prisms, with a third right-angled flint-glass prism between them in the manner shown. If a beam of white light *LT* falls upon the surface of the first prism *AB*, it is decomposed into its colored constituents, as shown by the divergent broken lines. In their passage through the prism the red rays are refracted least and emerge at *r*; the yellow rays emerge at *y*; and the violet rays, which are refracted most, emerge at *v*. If the light emerging from the prism *ABDE* now enters a second prism *A'B'D'E'* similarly placed to the first prism (their refracting edges *A* and *A'* being

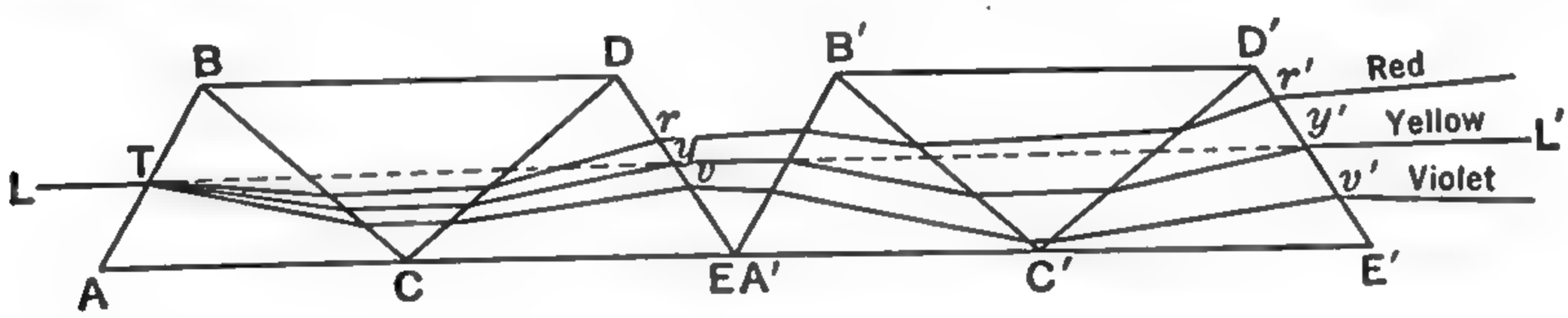
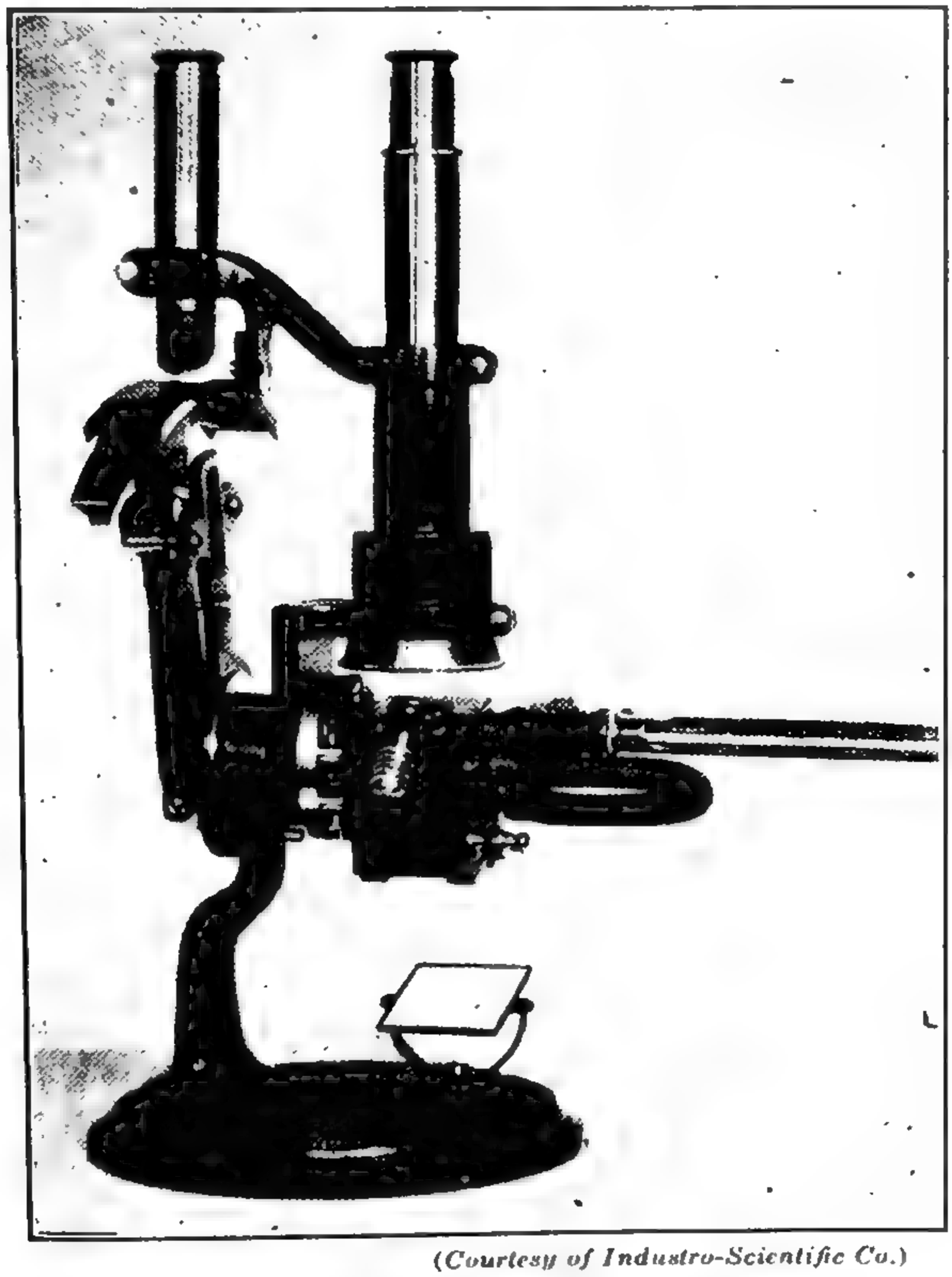


FIG. 46. Illustrating principles of compensator.

parallel and on the same side of the optical axis *LL'*), the colored rays will emerge from the second prism at the points *r'*, *y'*, and *v'*, respectively, the angle of dispersion for any two differently colored rays being twice that for the single prism *ABDE*.

If the two Amici prisms are now rotated in opposite directions around the optical axis *LL'*, the dispersion of the compensator will diminish until, when each prism has rotated 90° (the difference from the previous position being 180°), the dispersions of the two prisms neutralize one another and the dispersion of the compensator is zero. In this position the refracting edges *A* and *A'* of the two prisms will again be parallel, but on *opposite* sides of the optical axis *LL'* If we now imagine the direction of the colored rays through the two prisms to be reversed, we have an exact representation of the work performed in the compensator. The band of colored light from the double prism of the refractometer, passing in the direction *L'L*, emerges at *T* as a colorless beam, and the bright and dark halves of the field are sharply divided. By rotating the screw head the compensator can be given an equal but opposite dispersion to that of the liquid examined for any value from zero up to twice the dispersion of a single Amici prism.

After the compensator is set to the point where the colored bands disappear, the reading of the scale upon its drum (Z, Fig. 44) enables one to calculate the dispersion of the liquid examined for the F and C rays of the spectrum, the mean dispersion $n_F - n_C$ (difference in refractive index for the F and C rays) being determined with the help of a special table supplied with the instrument.



(Courtesy of Industro-Scientific Co.)

FIG. 47. Valentine precision refractometer.

Duplicate readings upon the Abbe refractometer with a sharp definition of the borderline should agree within two places of the fourth decimal. After each determination the prisms should be cleaned with wet filter paper and then wiped dry with a piece of soft linen.

A precision refractometer of the Abbe type has been designed by Valentine (Fig. 47). It is equipped with a sector scale divided to

the fourth decimal place of the refractive index, and with a compound microscope by which units of the fifth decimal place may be estimated. The accuracy of reading is stated to be about three units of the fifth decimal place. The scale covers the range from $n = 1.33$ to $n = 1.53$; this includes all the usual sugar products.

A new model of this instrument, with mechanical improvements, has been placed on the market in 1940; its sensitivity is the same as in the model just described.

Several types of Abbe refractometer are furnished with a special sector plate from which sugar percentages may be read directly. Otherwise the sugar percentages corresponding to various refractive indices are found from tables (see p. 98).

Illumination of Abbe Refractometer. For illuminating the refractometer ordinary daylight may be used, in which case the instrument should not be placed in the direct light of the sun. Since, however, daylight (especially in winter) is of variable intensity, and upon dark days not strong enough for the examination of deep-colored solutions, it is better on the whole to use artificial light of constant intensity. An incandescent electric lamp or Welsbach gas burner is a convenient method of illumination. A large sheet of cardboard, placed in front of the instrument so as to shield the light from the upper prism and from the eye of the observer, will protect the field of vision from the disturbing influences of extraneous light and increase to a marked extent the sensibility of adjustment. Special illuminating devices for refractometers are furnished by the manufacturers. Certain colorimeter or microscope lamps may also be used conveniently.

The modern high-intensity electric sodium-vapor lamp (p. 233) is finding increased application in refractometry, because it does away with the necessity of a compensator to correct for the dispersion of white light. Instruments equipped with a sodium-vapor lamp are described on pp. 125–128. The sodium-vapor lamp is preferable also for refractometers with compensators, because a sharp dividing line is obtained even with dark-colored products (see p. 105).

Regulation of Temperature in Abbe Refractometer. The refractive index of sugar solutions, as of all other substances, varies with the temperature, the index decreasing as the temperature rises. It is therefore important in all refractometer work that the temperature be kept constant during the course of observation. In the Abbe refractometer shown in Fig. 44 water of constant temperature is allowed to circulate in the direction of the arrow through the metal casings which surround the prisms; a thermometer screwed into the upper casing indicates the temperature.

The temperature of the circulating water should preferably be that at which the instrument has been standardized. But if the room temperature is appreciably higher, it is better to use water at room temperature in order to avoid condensation of moisture on the prisms.

Refractometer Heaters and Water-Pressure Regulators. A convenient piece of apparatus for controlling the temperature of refractometers is the Zeiss spiral heater and water-pressure regulator. This apparatus shown in Fig. 48 consists of a constant-level reservoir *A* connected by rubber tubing to the water supply and attached to a sliding frame which can be adjusted to different heights. The water passes from the reservoir to the spiral heater, which is placed upon a

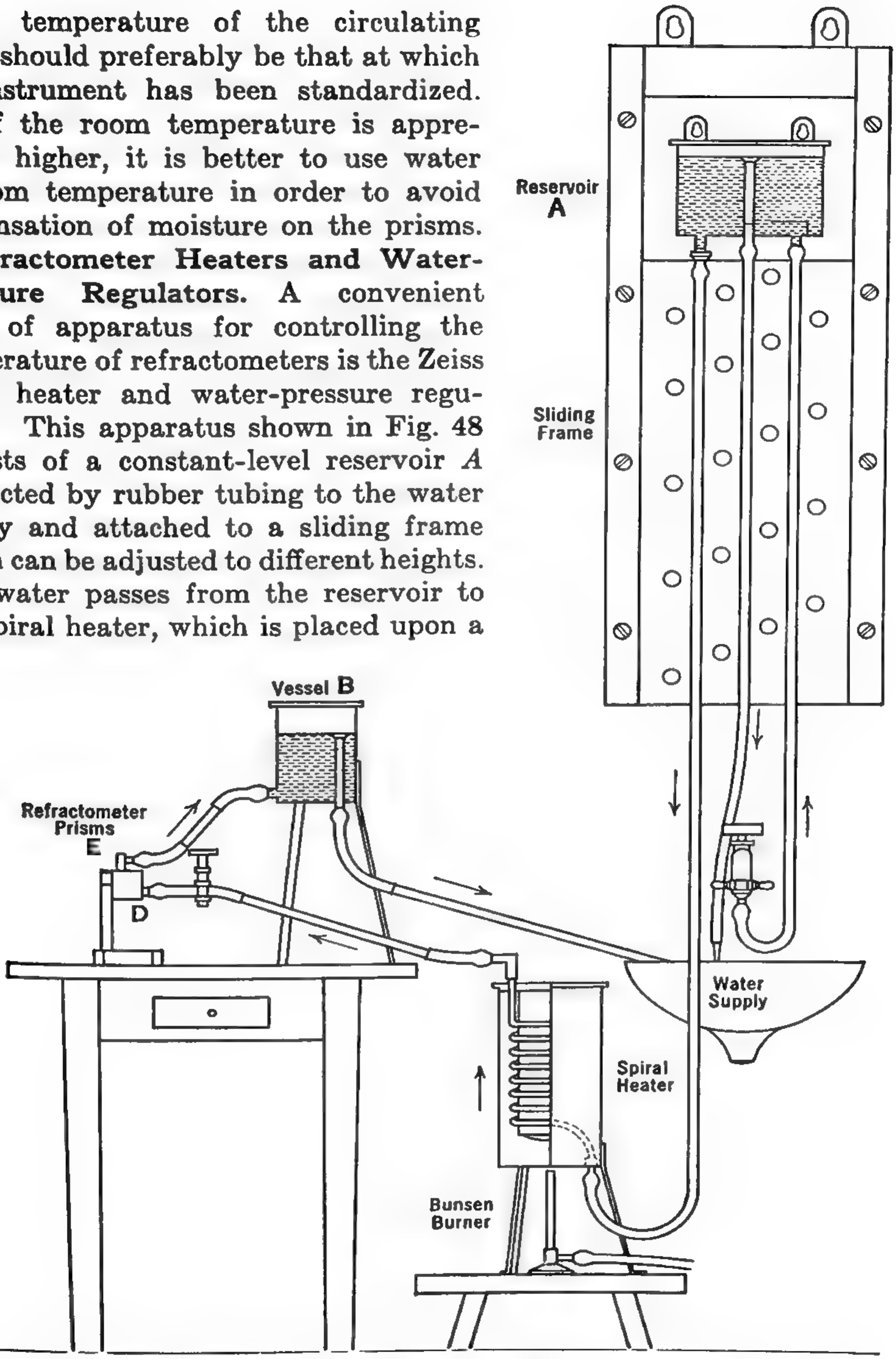
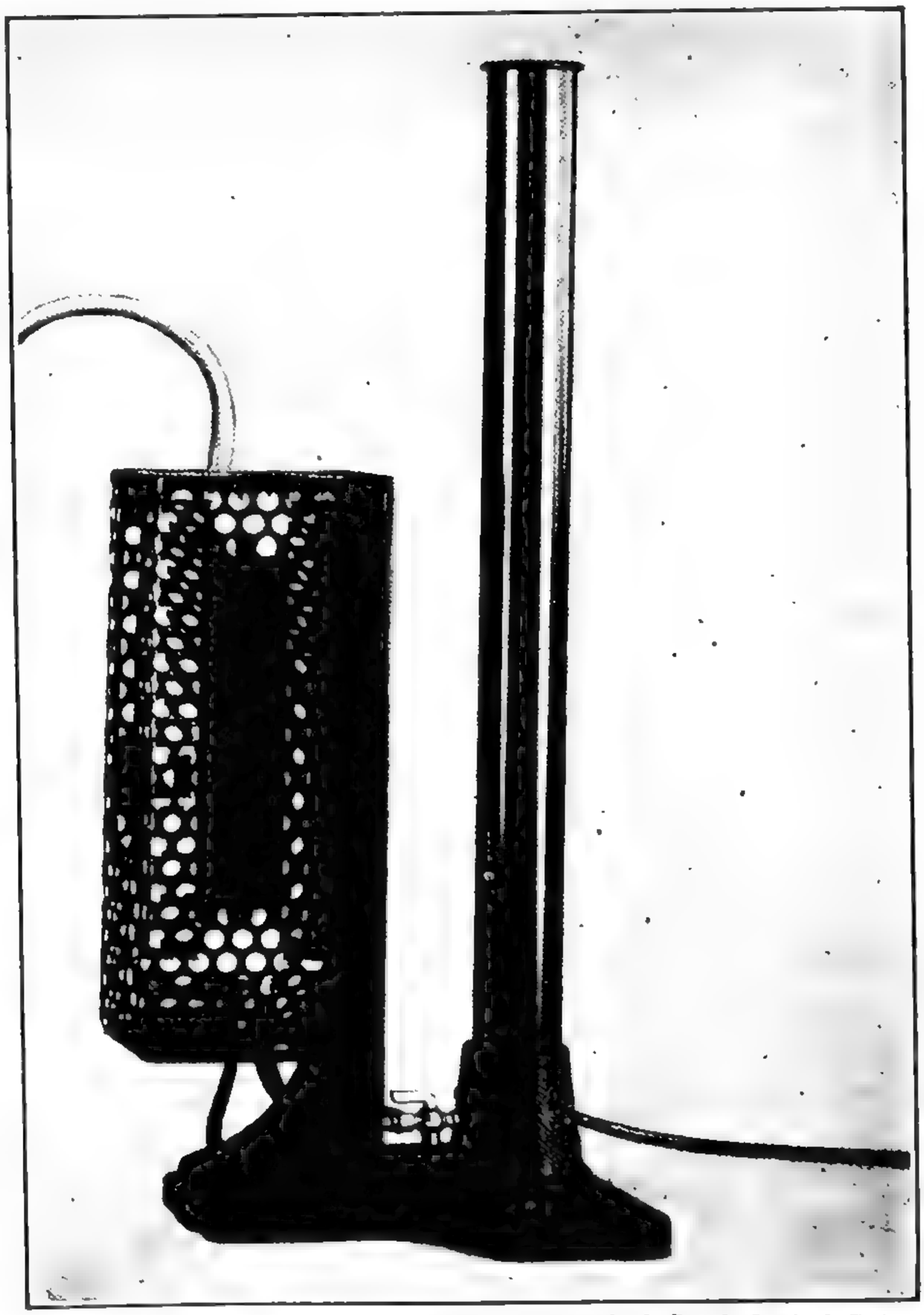


FIG. 48. Zeiss spiral water-heater with pressure regulator.

level below the refractometer. The heater consists of about 12 feet of copper tubing wound in a spiral and inclosed in a metal jacket which is heated by a Bunsen burner. The water flows from the heater upward to the prisms of the refractometer and thence to a constant-level vessel



(*Courtesy of Bausch & Lomb Optical Co.*)

FIG. 49. Bausch & Lomb electric temperature regulator.

B, from which the overflow escapes to a drain. The water, which should not flow too slowly, is first warmed to the approximate temperature by regulating the flame of the burner; the exact adjustment is then made by varying the speed of the flow, which is done by raising or lowering the pressure reservoir on its sliding frame. In this manner

the temperature can be maintained for hours within 0.1° C., provided of course that no variations take place in the temperature of the main water supply.

Instead of the Zeiss heater a large insulated heatable tank holding 50 to 100 liters of water may be used.

Another heater, requiring much less space than that just described, and also less time to bring the water to the desired temperature, is the electrical control apparatus of Bausch and Lomb, Fig. 49. It consists of a constant-pressure tower, from which the supply water flows first through an adjustable valve and from there through an electrical heater controlled by a rheostat. This apparatus furnishes water at 5° to 40° C. above the temperature of the input, within 0.1° C. up or down.

For very close temperature control the Höppler ultra-thermostat (p. 505) is recommended. It provides any desired temperature between —35° and +170° C., constant within about 0.02° C.

Testing the Adjustment of the Abbe Refractometer. The adjustment of the Abbe refractometer can be tested by means of liquids or glass test plates of known refractive power. Freshly distilled water free from air ($n_D^{20°} = 1.33299$) is convenient for testing the lower divisions of the sector scale; monobromonaphthalene ($n_D^{20°} = 1.658$) is convenient for testing the upper part of the scale; the latter substance unless freshly prepared usually requires to be redistilled (boiling point 277° C.). For checking intermediate points, Šandera and Mirčev[2] recommend glycol ($n_D =$ about 1.43) and glycerol diluted to about $n_D = 1.45$. The exact refractive index of these standards must be certified by an official testing bureau. The Abbe instrument is also usually supplied with a glass test plate whose index is marked upon the upper ground surface. The method of using the plate, which can be applied to any transparent solid, is that of grazing incidence (explained in detail under the immersion refractometer).

In using the test plate the instrument is reversed as shown in Fig. 50, the double prism spread open, and the polished surface of the plate attached to the upper prism by the capillary action of a drop of monobromonaphthalene; the polished end surface of the test plate is directed downwards to receive the reflected rays from the bright inner surface of the metal casing surrounding the lower prism. The average of several readings is taken, the prism being wiped clean and the plate reattached after each measurement. Care must be exercised not to confuse the reading in the reversed position of the sector scale. The average of the readings should not differ more than two points in the

[2] *Z. Zuckerind. čechoslovak. Rep.*, 63, 155 (1938/39).

fourth decimal from the value marked upon the plate. Should greater differences than this occur, the refractometer should be adjusted. In some of the instruments the adjustment is made by moving the index of the sector scale with a setpin until it corresponds to the value marked upon the test plate. The borderline of the field must remain meanwhile upon the intersection of the reticule, so that care must be exercised not to disturb the alidade while making the adjustment.

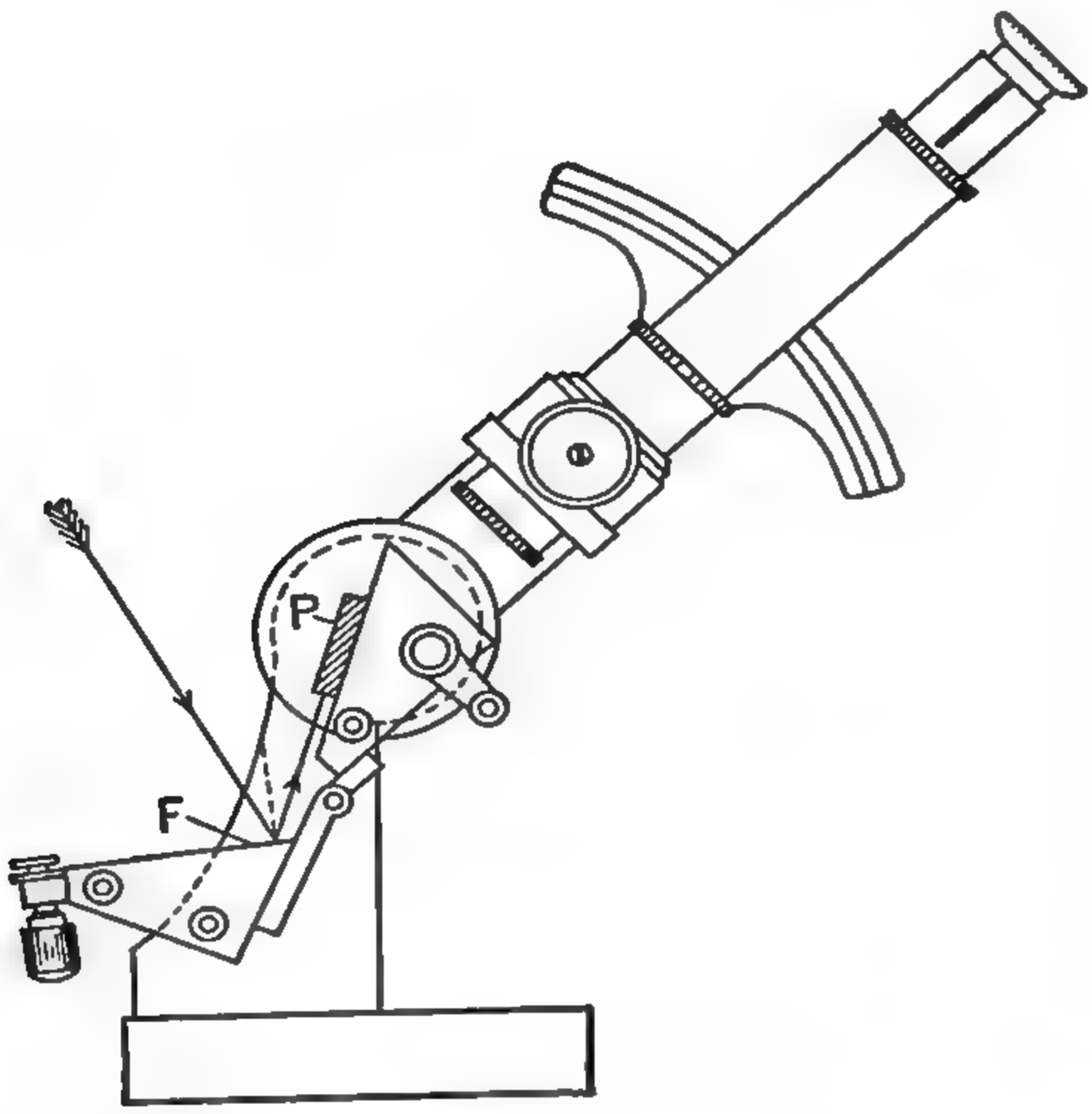


FIG. 50. Verifying adjustment of refractometer by test plate.

In later forms of the Abbe refractometer the adjustment is made by moving the reticule instead of the index. The process is the reverse of that previously described. The alidade is first moved until the index of the scale corresponds to the reading of the test plate; then by means of a key the screw *V* (Fig. 44), which moves the reticule, is turned until the intersection of the cross threads coincides with the borderline.

REFRACTOMETER TABLES FOR SUGAR SOLUTIONS

A number of tables have been constructed which give the refractive indices of sugar solutions for different concentrations. The first of such tables was published in 1883 by Strohmer,[3] who showed also that a fixed relation existed between the refractive index and specific gravity of sugar solutions. Using the method of least squares, Strohmer calculated this relation to be $n_D^{17.5°} = 1.00698 + 0.32717\ d$, in which d is the specific gravity of the solution at 17.5° C.

In 1901 Stolle,[4] using a Pulfrich refractometer, constructed tables for sucrose, glucose, fructose, galactose, lactose, and raffinose, a comparison of which showed that but very little variation existed in the refractive index of solutions of different sugars for the same concentration. Table XXIV is made up from the observations of Stolle upon sucrose solutions of different concentrations.

[3] *Oesterr.-ungar. Z. Zuckerind.*, 12, 925 (1883); 13, 185 (1884).
[4] *Z. Ver. deut. Zucker-Ind.*, 51, 469 (1901).

TABLE XXIV

Index of Refraction of Sugar Solutions

Concentration, grams to 100 ml.	Density (d) $\frac{17.5°}{4°}$	Per cent Sucrose in Solution	Refractive index (n) 17.5°	Refractive constant $\frac{n^2-1}{(n^2+2)\,d}$
0.9979	1.00241	1.00	1.33465	0.20612
4.0073	1.01406	3.95	1.33889	0.20615
12.0052	1.04484	11.49	1.35044	0.20617
17.9385	1.06736	16.81	1.35891	0.20621
25.0120	1.09420	22.87	1.36891	0.20617
35.0219	1.13194	30.94	1.38306	0.20610
45.8381	1.17246	39.10	1.39873	0.20619
55.0266	1.20651	45.61	1.41150	0.20602

The average value for the refractive constant (calculated by the formula of Lorenz and Lorentz) is 0.20614; from this it follows that the density (d) of sugar solutions may be calculated from the refractive index (n) by the equation

$$d_{4°}^{17.5°} = \frac{n^2 - 1}{(n^2 + 2) \times 0.20614}$$

According to later investigations by Landt[5] the refractive constant, or specific refraction, of sucrose, in concentrations up to 30 per cent, at 20° C., is the same as that of water, 0.2061. Above 30 per cent concentration the specific refraction decreases, becoming 0.2039 at 95 per cent concentration. It gradually approaches the value 0.203 for solid sucrose, showing that in concentrated solutions an association of sugar molecules takes place.

In 1906 Tolman and Smith,[6] using an Abbe refractometer, showed that "the refractometer is a satisfactory instrument for determining the soluble carbohydrates in solution under the same conditions as those under which specific gravity can be used, and in fact gives the same results; that it has many advantages over the specific-gravity method in speed, ease of manipulation, and amount of sample required for the determination," and that the refractometer can be used for a great deal of work where quickness and approximate accuracy only are necessary. Tolman and Smith give the following table showing index of refraction at 20° C. and percentage of various carbohydrates in solution.

[5] *Centr. Zuckerind.*, 43, 910 (1935).
[6] *J. Am. Chem. Soc.*, 28, 1476 (1906).

TABLE XXV

INDEX OF REFRACTION OF VARIOUS SUGAR SOLUTIONS OF DIFFERENT CONCENTRATION
(Dried in vacuum at 70° C. to constant weight.)

Index of Refraction, 20° C.	Sucrose	Maltose	Commercial Glucose	Lactose	Dextrin
	per cent	per cent	per cent	per cent	per cent
1.3343	1.00	1.00	1.00	1.00	1.00
1.3357	2.00	2.07	2.00	2.00	1.93
1.3402	5.00	5.07	5.00	5.13	4.87
1.3477	10.00	10.07	10.07	10.13	9.60
1.3555	15.00	15.12	15.06	15.13	14.13
1.3637	20.00	20.17	20.06	.....	18.94
1.3722	25.00	.....	25.00	.....	23.71
1.3810	30.00	.....	30.02	.....	28.78
1.3902	35.00	.....	35.03	.....	.....
1.3997	40.00	.....	40.05	.....	.....
1.4096	45.00	.....	45.04	.....	.....
1.4200	50.00	.....	50.03	.....	.....
1.4306	55.00	.....	55.02	.....	.....
1.4419	60.00	.....	60.01	.....	.....
1.4534	65.00	.....	65.01	.....	.....
1.4653	70.00	.....	70.00	.....	.....
1.4776	75.00	.....	75.00	.....	.....
1.4903	80.00	.....	80.00	.....	.....
1.5034	85.00	.....	85.00	.....	.....
1.5170	90.00	.....	90.00	.....	.....

It will be seen from the above table that dextrin alone of the carbohydrates examined differs appreciably from sucrose in its index of refraction. Comparing the density $\frac{20°}{4°}$ of the above sucrose solutions with their refractive indices the method of least squares shows that $n_D^{20°} = 0.9509 + 0.3818\, d_{4°}^{20°}$.

Tolman and Smith also studied the effects of temperature upon the refractive index of sugar solutions, and their results "show that the temperature correction for the specific gravity and the index of refraction are practically the same, and the table as given for Brix can be used for the index of refraction. The manner of using the table is the same. The reading of index of refraction is made at room temperature and this reading calculated to per cent of sugar, then the proper correction from the table calculated and applied."

Following the work of Tolman and Smith was that of Main[7] in 1907. Main was the first to demonstrate the practical utility of the Abbe refractometer in sugar-house work, and showed that the refractive index was an accurate measure of the moisture and total solids in all refinery products except the very lowest. The table of Main was

[7] *Intern. Sugar J.*, 9, 481 (1907).

employed very generally at one time by sugar chemists but has since been superseded by later ones. In 1911 Schönrock[8] measured the refractive indices of sugar solutions at the Physikalisch-Technische Reichsanstalt of Germany with the highest degree of refinement, and published a table giving the refractive indices to the fourth decimal place, for concentrations up to 66 per cent of sucrose. The new table was quickly accepted, but for concentrations above 66 per cent the table of Main was retained, although there is a divergence of 0.0003 re-

TABLE XXVI

Refractive Indices of Sugar Solutions at 20° C. According to Different Authorities

Per Cent Sugar	Tolman and Smith	Main	Schönrock	Krüss	Schulz	Landt
0.00	1.3330	1.3330	1.3330	1.3330	1.33302	1.33299
5.00	1.3402	1.3400	1.3403	1.3403	1.34037	1.34027
10.00	1.3477	1.3475	1.3479	1.3479	1.34795	1.34783
15.00	1.3555	1.3554	1.3557	1.3557	1.35578	1.35567
20.00	1.3637	1.3637	1.3639	1.3639	1.36389	1.36384
25.00	1.3722	1.3721	1.3723	1.3724	1.37232	.....
30.00	1.3810	1.3810	1.3811	1.3812	1.38109	.....
35.00	1.3902	1.3902	1.3902	1.3904	1.39022	.....
40.00	1.3997	1.3997	1.3997	1.3999	1.39972	.....
45.00	1.4096	1.4095	1.4096	1.4098	1.40961	.....
50.00	1.4200	1.4201	1.4200	1.4202	1.41994	.....
55.00	1.4306	1.4304	1.4307	1.4310	1.43060	.....
60.00	1.4419	1.4419	1.4418	1.4421	1.44171	.....
65.00	1.4534	1.4535	1.4532	1.4536	1.45322	.....
70.00	1.4653	1.4651	.....	1.4656	1.46513	.....
75.00	1.4776	1.4774	.....	1.4780	1.47744	.....
80.00	1.4903	1.4901	.....	1.4907	1.49012	.....
85.00	1.5034	1.5033	.....	1.5038	1.50316	.....
90.00	1.5170	.....	.....	1.5174	1.51656	.....
95.00	.....	.....	.....	1.5313	1.53026	.....

fractive index at that point between the two sets of data. Krüss[9] in 1920 and Schulz[10] in 1921 made evaluations, by the method of least squares, of the previous results of Main, Schönrock, and other observers. A comparison of the refractive indices of sucrose solutions according to different authorities is given in Table XXVI.

New measurements, to the fifth decimal place, were made by Landt[11] in 1933 upon solutions containing up to 24 per cent sucrose, and, when

[8] *Z. Ver. deut. Zucker-Ind.*, **61**, 421 (1911).
[9] *Z. Ver. deut. Zucker-Ind.*, **70**, 617 (1920).
[10] *Z. Ver. deut. Zucker-Ind.*, **71**, 347 (1921).
[11] *Z. Ver. deut. Zucker-Ind.*, **83**, 692 (1933).

the results were compared with those recalculated from the original data of Schönrock, it was found that they checked within one unit of the fifth decimal place. Landt's new values were adopted at the Ninth Session of the International Commission for Uniform Methods of Sugar Analysis, 1936. It was also decided to retain Schönrock's original table, to four decimal places, for concentrations from 25 to 66 per cent. From that point on, Schönrock's values have been extrapolated as far as 70 per cent, where the result checks that found experimentally by Main. Above 70 per cent the table of Main is to be used. The new table,[12] for concentrations from 0 to 85 per cent, based on these recommendations, is reproduced in the Appendix, Table 6.

Temperature Corrections. If the refractometer observations are made at other temperatures than 20° C., a correction must be applied in order to obtain the true value at the temperature of standardization. Staněk[13] prepared a chart of temperature corrections for the table of Main, assuming, like Tolman and Smith, that the variation in the refractive index is due solely to the effect of temperature on the volume of the solution. This assumption has been found to be erroneous, and the correction table of Staněk has been discarded in favor of more accurate figures. The correction of the refractometer readings of sugar solutions for changes in temperature has been subjected to a careful analysis by Schulz.[14] At the Ninth Session of the International Commission for Uniform Methods of Sugar Analysis it was decided to replace the Staněk table by a new one based on the original measurements of Schönrock. This table,[15] which disregards the effect of temperature on the refractive index of the measuring prism, is shown in the Appendix, Table 7. The corrections in this table agree closely with those given by Schulz.

Refractive-Index Table for Use in the Tropics. In order to minimize the temperature corrections when refractometers are used in tropical countries, Prinsen Geerligs[16] established a table showing the refractive indices of sugar solutions, containing up to 90 per cent sucrose, at 28° C., the normal average temperature in Java, and also a table of temperature corrections to be used when the readings are taken with an instrument calibrated at 28°. These tables were used for many years, but were superseded in 1936 by tables calculated by Landt[17] from the new tables for 20° C. The tables of Landt, for 28° C., were

[12] *Deut. Zuckerind.*, **61**, 997 (1936).
[13] *Z. Zuckerind. Böhmen*, **33**, 153 (1908/9).
[14] *Z. Ver. deut. Zucker-Ind.*, **71**, 88 (1921).
[15] *Deut. Zuckerind.*, **61**, 1026 (1936).
[16] *Intern. Sugar J.*, **10**, 69 (1908).
[17] *Deut. Zuckerind.*, **61**, 1026 (1936).

also adopted by the International Commission and are reproduced in the Appendix, Tables 6 and 8.

Refractive Indices of Solutions of Other Sugars. Stolle showed (p. 98) that the refractive indices of solutions containing the same percentages of various sugars do not differ very much. But it has since been found that the differences become progressively greater as the concentration increases. According to the most reliable data the refractive indices of 10 per cent solutions of sucrose and fructose differ by only 18 units in the fifth decimal place, corresponding to about 0.1 per cent sugar, but at 80 per cent concentration the difference is 5 units of the third decimal place, equal to about 2 per cent of sugar. This shows that the refractive index tables for sucrose cannot be used for ascertaining the concentration of a heavy fructose sirup without serious error.

The refractive indices of fructose solutions, containing up to 95 per cent of this sugar, have been measured by Jackson and Mathews,[18] who give the following formulas correlating n_D with the percentage by weight p:

$$n_D^{20} = 1.33300 + 0.0014159\,p + 0.00000491\,p^2 \quad (p = 0 \text{ to } 20)$$
$$n_D^{20} = 1.33344 + 0.0013625\,p + 0.000006645\,p^2 \quad (p = 20 \text{ to } 63)$$
$$n_D^{20} = 1.33377 + 0.0013570\,p + 0.000006680\,p^2 \quad (p = 63 \text{ to } 90)$$
$$n_D^{25} = 1.33252 + 0.0014059\,p + 0.00000487\,p^2 \quad (p = 0 \text{ to } 20)$$
$$n_D^{25} = 1.33312 + 0.0013415\,p + 0.000006762\,p^2 \quad (p = 20 \text{ to } 63)$$
$$n_D^{25} = 1.33345 + 0.0013360\,p + 0.000006800\,p^2 \quad (p = 63 \text{ to } 90)$$

The table computed from these data, giving also the change in the refractive index with 1° C. change in temperature, is shown in the Appendix, Table 9. The refractive indices have been calculated to the fifth decimal place for p up to 63, and beyond that to the fourth decimal place. They are consistently lower than those of sucrose solutions of the same concentration. For the same refractive index, the concentration of fructose solutions is from 1.5 to 2.5 per cent of the concentration higher than that of sucrose solutions. For example, a refractive index of 1.4158 at 20° C. indicates 48.0 per cent sucrose, but 48.82 ($48 + 48 \times 0.017$) per cent fructose.

Reliable refractive-index tables for solutions of glucose are not available as yet. According to Stolle's measurements at 17.5° C. the refractive indices of glucose solutions are lower than those of sucrose solutions of the same concentration, but Pulvermacher's results[19] at 25° C. show higher refractive indices for glucose solutions than the

[18] *Bur. Standards J. Research*, **8**, 403 (1932).
[19] *Z. anorg. allgem. Chem.*, **113**, 141 (1920).

accepted values for corresponding sucrose solutions, as may be seen from the following table:

VALUES OF STOLLE AT 17.5° C.

Per cent sugar	5	10	15	20	25
$n_D^{17.5}$, glucose	1.34030	1.34786	1.35555	1.36353	1.37169
$n_D^{17.5}$, sucrose	1.34054	1.34811	1.35594	1.36411	1.37256

COMPARISON OF PULVERMACHER'S RESULTS FOR GLUCOSE WITH SCHÖNROCK-LANDT VALUES FOR SUCROSE, AT 25° C.

Per cent sugar	1.00	2.11	4.36	10.20	15.72	20.14	24.03
n_D^{25}, glucose	1.3351	1.3366	1.3401	1.3486	1.3575	1.3646	1.3710
n_D^{25}, sucrose	1.3340	1.3356	1.3388	1.3476	1.3562	1.3634	1.3700

This subject requires further investigation, and the work of Stolle and of Pulvermacher on galactose, lactose, maltose, and raffinose also needs to be revised.

No precision measurements have been reported as yet of the refractive indices of invert-sugar solutions. Staněk and Vondrák,[20] Schneller,[21] Macara,[22] and de Whalley[23] have shown that they are lower than those of sucrose solutions of the same concentration. According to de Whalley the percentage of sucrose indicated by the refractometer must be increased 0.022 for each per cent of invert sugar present in the solution. If, for example, a sugar sirup contains 30 per cent of sucrose and 45 per cent invert sugar, its total concentration found by the refractometer, and expressed as sucrose, must be corrected by adding 45 × 0.022, or 0.99. De Whalley also found that the refractive indices of equimolecular mixtures of glucose and fructose are different from those of invert-sugar solutions of the same concentration but prepared from sucrose by hydrolysis with invertase or acid. The correction factor for the former is only 0.007 to 0.009. This subject also requires further investigation.

Mutarefraction. The discrepancies in the observed refractive indices of solutions of reducing sugars are probably due largely to a phenomenon related to mutarotation. Stolle first noted that the refractive indices of solutions of certain sugars changed upon standing. Riiber[24] found that mutarotation is accompanied not only by a change

[20] *Z. Zuckerind. čechoslovak. Rep.*, **45**, 203 (1920/21).
[21] *J. Assoc. Official Agr. Chem.*, **9**, 156 (1926).
[22] *Analyst*, **56**, 391 (1931).
[23] *Intern. Sugar J.*, **37**, 353 (1935).
[24] *Ber.*, **56B**, 2185 (1923); **57B**, 1599 (1924); **58B**, 737 (1925).

in volume of the solution, but also by an independent change in the refractive index. All three are caused by the formation of an equilibrium mixture from the isomer or isomers present in the freshly prepared solution. The refractive indices of some solutions measured by Riiber are as follows:

Sugar	n_D^{20}, 10 Per Cent Solution
α-glucose	1.34776
$\alpha \rightleftarrows \beta$-glucose	1.34785
β-glucose	1.34790
β-fructose	1.34791
$\alpha \rightleftarrows \beta$-fructose	1.34762

Estimation of Solids in Technical Sugar Products with the Abbe Refractometer

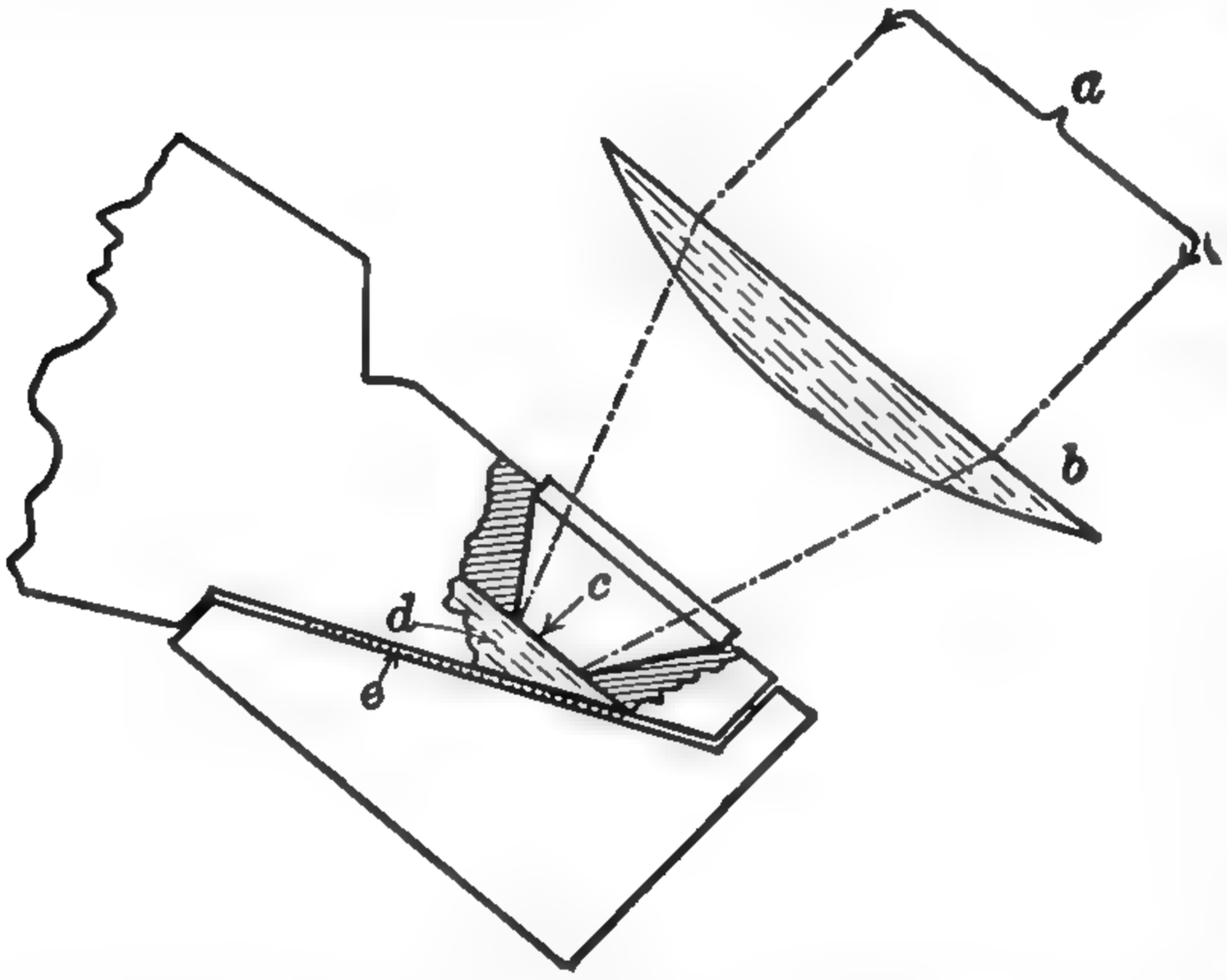
The use of the Abbe refractometer was extended to raw sugar-cane products by Prinsen Geerligs and van West,[25] and its application to the analysis of sugar-beet products has been studied by von Lippmann, Hübener, Lange, and many others. For both types of products it was found that the refractometer gives values for solid matter more closely agreeing with the true dry substance than those based on the specific gravity.

Examination of Dark-Colored Sugar Solutions with the Refractometer. In the examination of dark-colored sugar solutions, molasses, sirups, extracts, etc., by means of the refractometer, it is not always possible for the compensator to eliminate completely the effects of dispersion; the borderline of the field is then more or less blurred and a sharp adjustment to the intersection of the reticule becomes a matter of some difficulty. In solutions which are not too strongly colored this trouble may be remedied by bringing the borderline to the point of intersection alternately from each side of the field; the average of the readings thus obtained will correct to a large extent the errors of faulty adjustment. Some authorities have recommended that with dark solutions the compensator be adjusted to a colored border, the color most sensitive to the observer's eye being selected; this, however, is not very satisfactory, and if the blurring of the borderline is excessive, recourse must be had to examination by reflected instead of transmitted light.

For this purpose most of the later models of the Abbe instrument

[25] *Arch. Suikerind.*, 15, 487 (1907).

are provided with a removable cover *C* (Fig. 44) over an opening in the upper prism *A*. The mirror *R* is first turned so that no light from it can enter the prisms, cover *C* is taken off, and a strong beam of light is directed to fall into the opening in the upper prism, a condensing lens being used if necessary. An effective lighting arrangement devised by Dědek[26] is shown in Fig. 51.



(*Reproduced from Z. Zuckerind. čechoslovak. Rep.*, *45*, *6*.)

FIG. 51. Dědek's lighting arrangement for determining refractive index by reflected light.

The course of the light in this case can be understood by referring again to Fig. 43, in which let *SF* be the upper surface of the film of molasses and *Lo*, Lo_1, etc., the beams of light passing through the side opening. For the reflected beams with an angle of incidence less than that for total reflection, as *Lo*, Lo_1, Lo_2, etc., where a part of the light is absorbed by refraction, there will be not a dark but a shaded field, while for the beams that are totally reflected, as Lo_4, Lo_5, etc., no light is absorbed by refraction and the field will be bright. The boundary line, when a measurement is made by reflected light, is therefore not between a bright and a dark field but between a bright and a shaded one. While the boundary line under these circumstances is not so pronounced as that produced by transmitted light under the best conditions, it is yet sufficiently sharp to test the darkest ordinary molasses without dilution and without the errors of measurement which result therefrom.

The accuracy of the readings may be increased still further by placing a piece of fine copper gauze (*e* in Fig. 51) in the space between the two prisms, as recommended by Dědek; this reverses the two halves of the field again.

The prism box of the Abbe refractometer made by Bausch and Lomb is provided with a combination shield and reflector which is swung open to permit measurements by reflected light.

Markovits[27] has reported the following comparative results of solids

[26] *Z. Zuckerind. čechoslovak. Rep.*, **45**, 1 (1920/21).

[27] *Louisiana Planter*, **76**, 90 (1926).

determinations by the refractometer and by drying on quartz sand at 70° C. in vacuo:

	Refractometer	Drying
	per cent solids	per cent solids
Cuban blackstrap	81.0	77.79
Cuban blackstrap	78.0	76.08
Cuban blackstrap	79.4	78.39
Cuban blackstrap	79.0	76.14
Cuban blackstrap	76.2	73.47
Cuban blackstrap	78.2	76.45
Cuban blackstrap	79.2	76.44
Refinery sirup	77.0	77.39
Refinery blackstrap	76.8	76.89
Refinery blackstrap	81.0	78.29
Refinery blackstrap	80.0	79.39
Refinery blackstrap	81.6	78.43

All the raw-sugar blackstraps and some of the refinery blackstraps show considerably higher values by refractometer than by drying, but there is fair agreement between the two values for one refinery sirup and one refinery blackstrap.

Similar comparisons for beet molasses are cited from results published by Šandera:[28]

No.	Solids by Refractometer	Solids by Drying
1	80.6	81.60
2	80.2	81.80
3	81.0	81.24
4	79.5	81.48
5	77.3	78.36
6	74.9	76.20
7	74.6	76.14
8	82.0	83.16
9	77.8	79.36
10	78.6	78.74
11	79.1	79.92
12	76.3	77.32

Contrary to the results obtained with cane molasses, the refractometer values are consistently lower than those obtained by drying, but in a few instances the differences are well within the limit of error.

Products containing high percentages of invert sugar and little ash give lower results with the refractometer, using sucrose tables, than by drying in vacuo at 60° to 70° C., for reasons explained on p. 104. But when the refractometric solids are corrected for the invert-sugar

[28] *Z. Zuckerind. čechoslovak. Rep.*, 53, 1 (1928/29).

content of the products, close agreement is obtained, as shown by de Whalley[29] for two samples of Golden Syrup:

No.	Corrected Solids by Refractometer	Solids by Drying
1	83.84	83.97
2	78.04	78.07

If the Abbe refractometer is not equipped for measurements by reflected light, the color of the solution must be reduced by some method of dilution or clarification.

Dilution Method. Not only may this be used in the examination of dark-colored products, but it must be resorted to also when the material contains considerable quantities of sugar crystals, such as massecuites, sugars, etc., in which case all the soluble matter is dissolved with a known amount of water.

Example. Ten grams of massecuite was dissolved in 10 ml. of hot distilled water, the weight of the mixture after cooling to 20° C. being brought to 20 g. by addition of distilled water of 20° C. The refractive index of the mixture was 1.4107, which according to the table of Schönrock-Landt indicates 54.5 per cent water; 54.5 per cent of 20 g. = 10.90 g. water in mixture; 10.90 − 10 (g. water added) = 0.90 g. water in original massecuite, or 9.0 per cent.

Hardin has made comparative determinations of the moisture in different grades of sugar by drying and by the refractometer with the following results:

Grade of Sugar	Refractive Index, 20° C. (1 part sugar +1 part distilled water)	Per Cent of Water	
		By Refractometer	By Drying to Constant Weight
		per cent	per cent
Refined sugar	1.4200	0.10	0.05
Hawaiian centrifugal	1.4199	0.20	0.45
Philippine mats (dried out)	1.4197	0.40	0.82
Java centrifugal	1.4190	1.00	0.82
Louisiana centrifugal	1.4189	1.10	1.05
Cuban centrifugal	1.4181	1.90	1.93
Muscovado	1.4179	2.10	2.40
Molasses sugar	1.4172	2.70	2.83
Molasses sugar	1.4139	5.90	5.54

The variations in the results by the two methods are in both directions, and may have been due either to the presence of trash in the

[29] *Intern. Sugar J.*, **38**, 345 (1936).

sugar or to the influence of non-sugars. Since the refractometer indicates only the percentage of dissolved solids, any insoluble matter present in the weighed sample will introduce an error in the calculation.

On the other hand, if it is desired to ascertain only the dry substance in solution, the refractometer offers a great advantage over the densimetric method because filtration is usually not necessary. If suspended matter is present in large quantity it will darken the field of the refractometer and interfere with the adjustment of the borderline. The determination must then be made by reflected light, or the solution must be filtered.

In the dilution of impure sugar products with water an error will be introduced in the refractometer reading in the same manner as in the determination of specific gravity, owing to the difference in contraction between solutions of sugar and of the accompanying impurities (p. 78).

A study of the errors resulting from unequal contraction, when dilution is employed in densimetric and refractometric methods of analysis, has been made by Staněk.[30] Fifty per cent solutions of betaine and of various organic salts of sodium and potassium were prepared. These solutions were then diluted with known weights of water and the percentage of dry substance determined from the degrees Brix, from the refractive indices according to Main's table, and by drying on sand in a Soxhlet oven at 102° C. A few of the results are given in Table XXVII.

It will be noted from the table that the refractometer gives a much closer approximation to the true dry substance than the degrees Brix, the refractometer yielding usually lower results and the degrees Brix higher. It is also seen that the sodium salts of organic acids give higher results by both methods than potassium salts. Contraction upon dilution is noted invariably, the results corrected for dilution being higher according to the amount of water added. The usual effect of this contraction is to make the error in estimating non-sugars less by the refractometer and greater by degrees Brix. Neither of these methods for estimating non-sugars approaches in point of accuracy the method of actual drying.

The errors in determining the refractive index of dark impure sugar solutions, resulting from dilution with water, may be largely eliminated by employing the method of Tischtschenko,[31] which consists in reducing the color of the product by means of a solution of pure sucrose of about the same density as the liquid to be examined. The disturbing influences of color dispersion in the refractometer field

[30] *Z. Zuckerind. Böhmen*, **34**, 5 (1909/10).
[31] *Z. Ver. deut. Zucker-Ind.*, **59**, 103 (1909).

TABLE XXVII

Comparative Determinations of Solids by Brix, Refractometer, and Drying at 102° C.

Substance Taken	True Dry Substance	Dry Substance by		
		Degrees Brix	Refractometer	Drying at 102°
	per cent	per cent	per cent	per cent
Betaine (anhydrous)	5	2.2	5.10	5.05
	10	4.3	10.20	10.01
	25	10.8	24.15	25.03
Sodium formate	5	8.1	4.60	4.99
	10	15.6	8.85	10.04
	25	37.7	20.55	25.05
Potassium formate	5	7.3	3.60	5.00
	10	14.28	7.20	9.97
	25	35.7	17.20	25.09
Sodium acetate	5	6.7	5.00	4.97
	10	13.1	9.70	9.99
	25	31.1	22.70	25.00
Potassium acetate	5	6.6	5.00	5.00
	10	12.8	8.25	10.07
	25	30.4	19.75	25.15
Sodium butyrate	5	4.75	4.90	4.90
	10	9.4	10.25	9.89
	25	22.9	24.35	24.94
Sodium lactate	5	6.3	5.00	5.10
	10	12.3	10.00	10.07
	25	30.2	24.05	25.05
Potassium lactate	5	6.3	4.85	5.18
	10	12.5	9.10	10.13
	25	30.3	21.65	25.20
Sodium glutaminate	5	6.8	6.40	5.05
	10	13.2	12.50	10.23
	25	31.1	30.05	26.41
Potassium glutaminate	5	6.7	5.90	5.03
	10	13.1	11.50	10.24
	25	30.65	27.70	25.27

are in this way overcome without the errors of contraction. The method of operation is as follows: A known weight (a) of the molasses, sirup, etc., is intimately mixed with a known weight (b) of pure sugar solution, whose sugar content (p) has been previously determined by means of the refractometer. The refractive index of the mixed solution is then determined and the corresponding percentage (P) of dry substance found from the table. The percentage of dry substance (x) in

the molasses, sirup, etc., is then calculated by the formula $ax + bp = (a + b)P$, whence

$$x = \frac{(a + b)\,P - bp}{a}$$

Example. Weight of beet molasses (a) = 14.1028 g.
Weight of sugar sirup (b) = 13.2438 g.
Sugar in sirup (p) = 51.3 per cent
$n_D^{20°}$ of mixture = 1.4538 = 34.74 per cent water (Schönrock's table)
Solids of mixture (P) = 100 − 34.74 = 65.26 per cent

Substituting these values in the formula, x = 78.36 per cent solids in molasses. The method by water dilution gave 79.11 per cent. Direct determination by drying gave 77.80 per cent.

If a sugar sirup of greater density had been used for mixing, the value of x would have been closer to the result by direct determination.

TABLE XXVIII

COMPARATIVE DETERMINATIONS OF SOLIDS IN BEET MOLASSES BY DRYING, SPECIFIC GRAVITY, AND REFRACTOMETER

Number	By Direct Determination	By Degrees Brix	By Refractometer	
			Water Dilution	Tischtschenko's Method
1	76.78	78.90	77.90	76.80
2	77.95	79.80	78.50	78.00
3	76.22	78.60	77.00	76.10
4	77.85	79.30	78.60	77.90
5	77.05	79.40	78.20	77.30
6	77.55	79.20	78.10	77.80
7	77.97	79.90	78.60	78.30
8	77.32	79.30	78.20	77.70
9	77.50	79.30	78.60	77.88
10	77.31	79.60	78.40	77.70
11	76.58	78.90	77.70	77.00
12	76.94	79.20	77.90	77.40
13	77.43	79.60	78.50	77.90
14	76.53	78.90	77.70	77.00
15	77.82	80.00	79.00	78.30
16	77.90	80.20	78.90	77.40
Average....	77.29	79.38	78.24	77.53

If equal weights of molasses and sugar solution are used in Tischtschenko's method, then $a = b$ in the formula, whence $x = 2P - p$; the labor of calculation is thus considerably reduced. In using the method, the mixture of molasses and sugar solution must be perfectly homogeneous. Care must also be exercised, as always, that no air

bubbles are inclosed with the liquid between the prisms. A comparison of results in determining dry substance in different samples of beet molasses by various methods is given by Lippmann[32] in Table XXVIII.

It will be noted from the table that the average error of estimating dry substance in the 16 samples of beet molasses was, by degrees Brix, +2.09 per cent; by refractometer, using water dilution, +0.95 per cent; and by refractometer, using Tischtschenko's method, only +0.24 per cent.

For a typical sample of cane molasses Cross[33] reported 79.63 per cent dry substance by drying in vacuo at 70° C. The Brix was found 2.89 per cent higher. Determinations with the refractometer gave errors of: —0.23 per cent without dilution, +0.61 per cent by dilution with water, and +0.12 per cent by dilution with sugar solution.

Clarification Methods. Another method of correcting the disturbances in refractometer work due to color of solution is by clarification. Lead subacetate is the reagent most generally employed for this purpose. The use of this and similar salts must be limited, however, to the greatest possible minimum, since the excess of salt remaining in the clarified solution causes an increase in the refractive index. In the following experiments made by Rosenkranz[34] at the Berlin Institute for Sugar Industry, the effect of increasing the quantity of subacetate is shown upon the refractive index of a molasses containing 78.59 per cent dry substance and diluted 1:1, inclusive of the lead solution added.

Lead Subacetate	Specific Gravity, Dilute Solution, 20°	Calculated Brix of Original Molasses	Refractive Index, Dilute Solution	Dry Substance, Dilute Solution (Main's Table)	Calculated Dry Substance, Original Molasses
ml.					
....	1.1813	81.9	1.3994	39.85	79.70
5	1.1865	84.0	1.4003	40.3	80.60
10	1.1912	85.7	1.4009	40.6	81.2
12.5	1.1951	87.2	1.4022	41.3	82.6

Another material recommended by Lippmann for decolorizing dark sirups, etc., for the refractometer is " Decrolin," the zinc salt of formaldehyde sulfoxylic acid, $CH_2OH{\cdot}O{\cdot}SO{\cdot}Zn{\cdot}OH$. One to two per cent of Decrolin is used and the liquid heated to about 55° C. to hasten solution and decolorization.

[32] *Deut. Zuckerind.*, **34**, 402 (1909).
[33] *Louisiana Bull.*, **135**, 10 (1912).
[34] *Z. Ver. deut. Zucker-Ind.*, **58**, 195 (1908).

For the refractometric examination of turbid beet juices, etc., Herzfeld[35] has recommended the addition of a few drops of 10 per cent acetic acid, heating for 2 minutes at 80° C. to coagulate albuminoids, and filtering. With beet juices the effect of dilution (1 to 5 per cent) is compensated by the greater refractive index of the 10 per cent acetic acid used, as shown in the following experiment:

10 ml. Beet Juice	Refractive Index, $n_D^{20°}$	Dry Substance by Main's Table
+0.5 ml. water	1.3583	16.75
+0.5 ml. acetic acid (10 per cent)	1.3595	17.45
+0.25 ml. water	1.3588	16.95
+0.25 ml. acetic acid (10 per cent)	1.3591	17.20
+0.10 ml. water	1.35905	17.15
+0.10 ml. acetic acid (10 per cent)	1.35905	17.15

Determination of Fine Grain in Molasses. *Kalshoven's Method.* Molasses usually contains a certain quantity of fine sugar crystals which have either passed through the centrifugal screen or have formed subsequently during storage. Kalshoven[36] devised a method for estimating this fine grain. The dissolved solids in the molasses, exclusive of the crystals, are determined directly by means of the refractometer. In another portion of the molasses the crystals are dissolved by dilution with water, and the solids are again determined with the refractometer. From these data the percentage of crystals can be calculated as shown below.

The Java Sugar Experiment Station uses the Kalshoven method in the following form.[37] About 100 g. of the molasses is transferred to a bottle. Another 100 g. of the molasses is weighed accurately into a second bottle; 5 or 10 g. of water (depending on the amount of fine grain present) is added and thoroughly mixed with the molasses. Both bottles are placed in a thermostat and slowly revolved until the crystals in the diluted molasses are completely dissolved, as shown by microscopic examination. This usually requires from 18 to 24 hours. The refractive indices of the original and of the diluted molasses are then determined by reflected light (p. 105). The refractometer solids in the diluted molasses are corrected for dilution by multiplying by 1.05 (5 per cent dilution) or 1.1 (10 per cent dilution), and the result is designated by p. If the refractometer solids in the original

[35] *Z. Ver. deut. Zucker-Ind.*, 58, 197 (1908).
[36] *Arch. Suikerind.*, 27, 1560, 1700 (1919).
[37] "Methoden van Onderzoek," 6th ed., p. 363, 1931.

molasses are termed q, then the percentage of fine grain x is found by the formula:

$$x = \frac{100\ (p - \ q)}{100 - q}$$

Example. The refractometer solids, expressed as sucrose, in the undiluted molasses are found to be 86.6 (q), and in the molasses diluted with 10 per cent water 79.5. The corrected p is 79.5×1.1, or 87.45. Then the percentage of fine grain x equals $100\ (87.45 - 86.6)/(100 - 86.6) = 6.3$.

It is readily seen that small errors in the determination of the refractive index may cause large deviations in the percentage of fine grain. The method has been criticized on other grounds, also. Both Dědek and Schoorl have found that perfectly grain-free molasses, when examined by Kalshoven's method, may show apparent grain contents of several per cent, owing to the effect of dilution on the refractive indices of the salts and organic impurities present. Even Tischtschenko's method of dilution with sucrose solution does not eliminate this error.

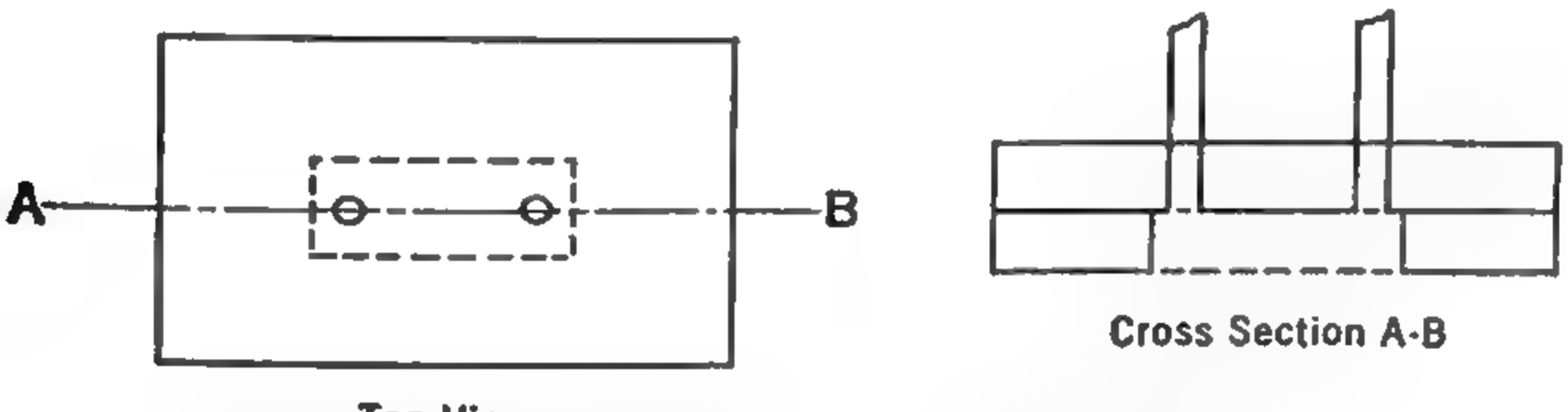
Dědek's Method. In order to obtain correct results, Dědek[38] dissolves the fine grain not by dilution but by heating the molasses in a small metal autoclave immersed in a glycerin bath at 100° or 110° C. for 15 to 30 minutes. A small metal cylinder acting as plunger is placed in the autoclave tube so that the molasses can be thoroughly mixed by removing the apparatus from the bath several times during the heating period and turning it up and down. The autoclave is then quickly cooled under running water, again being turned up and down until the molasses becomes so stiff that the plunger no longer moves freely. The autoclave is opened, and the refractive index of the molasses determined. This gives p in the above formula directly, and the percentage of grain is calculated as in the dilution method. Šandera[39] found that this method, though somewhat tedious, gives satisfactory results.

Other Uses of the Abbe Refractometer. Thieme[40] observed that a grain-free molasses, spread out in a thin film in dry air, loses its water without crystallization taking place, and dries to a glassy skin. This property makes it possible to determine the vapor-tension curve of the molasses with the refractometer. If any grain is present in the original molasses, it is dissolved by adding a little water and mixing thoroughly. The lower prism on the Abbe instrument is removed,

[38] *Z. Zuckerind. čechoslovak. Rep.*, **45**, 1 (1920/21).
[39] *Z. Zuckerind. čechoslovak. Rep.*, **54**, 389 (1929/30).
[40] *Arch. Suikerind.*, **41**, I, 325 (1933).

and a thin film of the molasses is spread on the surface of the upper prism. A small glass beaker, about 2.5 cm. in diameter, the upper edge of which has been ground flat and smeared with a little desiccator grease, is attached to the upper prism by means of a rubber band. The beaker is filled successively with mixtures of sulfuric acid and water of known vapor tension, and after equilibrium is reached the concentration of the film is measured by means of reflected light (p. 105). The vapor-pressure isotherm is obtained by plotting the refractometer solids against the relative humidity indicated by the vapor pressure of the sulfuric acid-water mixtures. Conversely, after the isotherm for a certain molasses has once been determined, the curve can be used to measure the relative humidity of the air by finding the refractometer solids in the molasses film exposed to the air.

It is also possible to dry the molasses film completely and to determine the refractometer solids of the dry substance by placing, instead of the beaker, a drying chamber over the upper prism of the



(*Reproduced with permission from Arch. Suikerind. 41, I, 328.*)

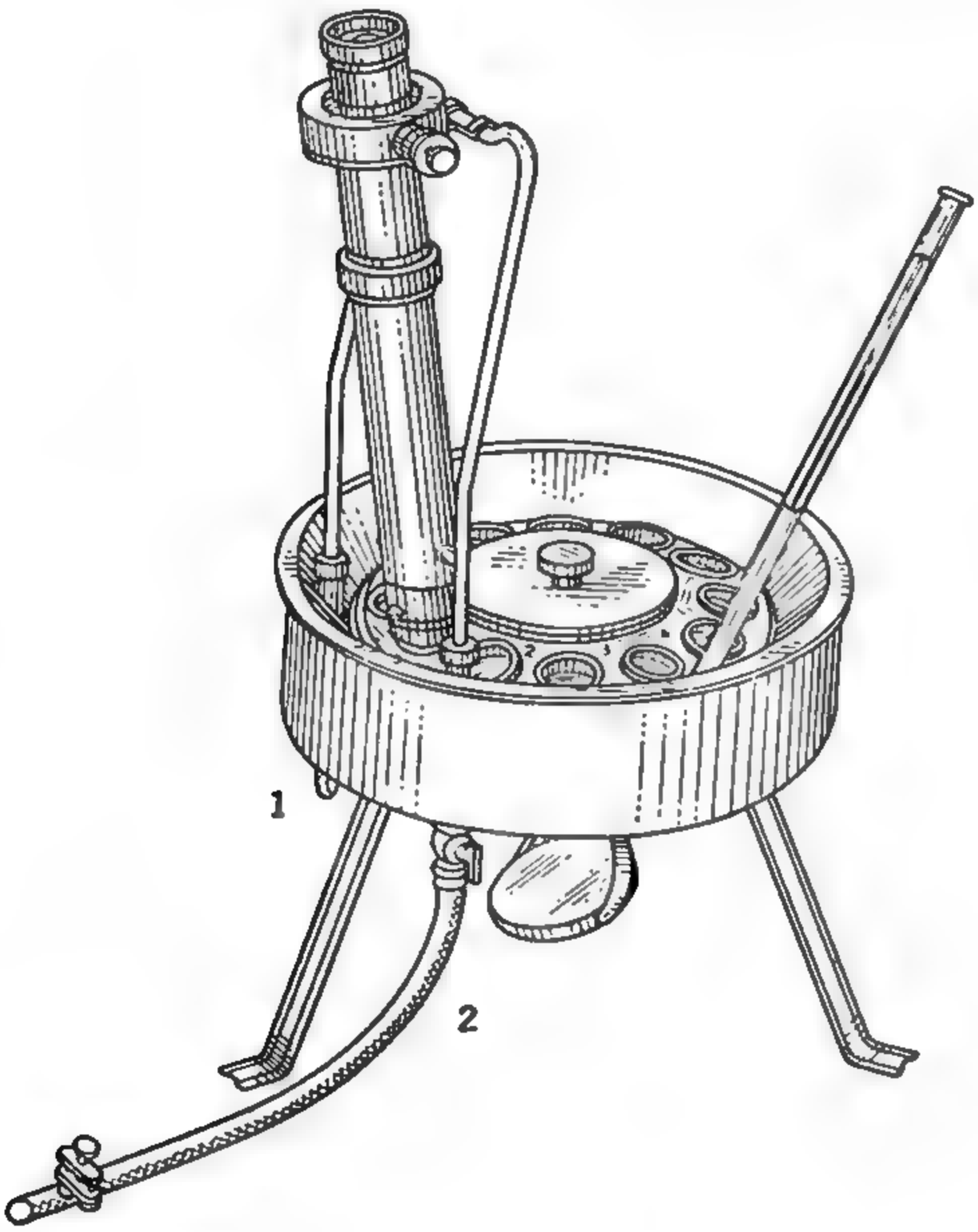
FIG. 52. Thieme's drying chamber for determining total solids in molasses.

Abbe refractometer. Two rectangular pieces, 3.5 by 5.5 cm., are cut from sheet rubber about 7 mm. thick. In one of these plates a rectangular opening, 1.2 by 2.5 cm., is cut out, and in the other plate two small circular holes are bored and short pieces of glass tubing inserted. The two plates are cemented together to form a chamber, as shown in Fig. 52. A film of the molasses to be examined is placed on the upper prism of the refractometer, and the chamber fastened over the prism with rubber bands. The film is dried either by passing warm air over it or by applying a vacuum. The refractive index of the water-free molasses is read, and converted into dry substance by extrapolation of the Landt-Schönrock table. The figure thus obtained may then be used to correct the refractometer solids found in the original molasses. Supposing that the latter were found to be 84.68, and the refractometer solids in the dry substance were 100.75, the dry substance in the original molasses was $84.68 \times 100/100.75 = 84.05$. This figure is only an approximate measure of the dry

substance because the volume change between 100 per cent and 84 per cent dry substance is neglected.

The Immersion Refractometer

A second form of instrument which is used for determining the refractive power of sugar solutions is the immersion refractometer, the Zeiss model of which is shown in Fig. 53. It is furnished with ten interchangeable prisms, each of which covers an interval of about three to four units of the second decimal place of the refractive index, with sufficient overlapping to cover an unbroken range from n_D 1.325 to 1.647. Only the first six prisms, reading up to n_D 1.5322, are required for the analysis of sugar products. A special prism, marked *Z*, may also be obtained, for the range from n_D 1.331 to 1.372, that is, up to about 25 per cent of sucrose. This prism has been specially designed for Bachler's "one-solution" method of purity determination,[41] in which a normal weight (26.000 or 26.026 g.) solution of any factory product, from juice to molasses, is prepared and used for the determination of the refractive index, and after clarification with dry lead subacetate, for that of the polarization.



(*Courtesy of Carl Zeiss, Inc.*)

FIG. 53. Zeiss immersion refractometer.

The Bausch and Lomb immersion refractometer is furnished with six interchangeable prisms, covering the range from n_D 1.325 to 1.544. With the exception of the first one, the range of the individual prisms is somewhat different from the corresponding Zeiss prisms.

The immersion refractometer gives a much sharper borderline, thus allowing a greater magnification in the telescope, with a correspond-

[41] *Facts About Sugar*, **28**, 420 (1933); see also Vondrák, *Z. Zuckerind. čechoslovak. Rep.*, **52**, 381 (1927/28); Dolínek, *Z. Zuckerind. čechoslovak. Rep.*, **54**, 627 (1929/30).

ing increase in the accuracy of observation. In the immersion refractometer there is no sector; the scale is placed below the eyepiece of the telescope, the latter, unlike the Abbe refractometer, being rigidly connected with the prism holder.

The principle of the immersion refractometer is the same as that of the Abbe instrument, being based upon an observation of the borderline of total reflection. In Fig. 54, G is a cylindrical glass prism with its refracting surface DE immersed in the liquid W contained in the glass beaker V. If we suppose light to pass through the top of the prism from the surface AB, the parallel rays sP, $s'P'$, $s''P''$, etc., will be refracted in the liquid in the direction PM, $P'M'$, $P''M''$, etc. By increasing the angle of incidence for the parallel rays upon the surface DE, a point is reached where the parallel rays rP, $r'P'$, $r''P''$, etc., are refracted along the surface of the prism towards D. This is the borderline of total reflection as explained under Fig. 43, where the angle of refraction is 90°. In the use of the immersion refractometer the course of the light is in the reversed direction to that just described, the light being reflected from the mirror HK through the bottom of the beaker V so as to pass as nearly parallel as possible to the oblique surface of the prism. The rays of light which coincide with the surface DE form the borderline for total reflection and are refracted upward through the prism as the parallel rays Pr, $P'r'$, $P''r''$, etc., which being condensed by the objective O of the refractometer telescope upon the point x of the scale S, form the borderline for observation; the rays of light which may strike the prism surface obliquely, as MP, $M'P'$, $M''P''$, etc., are refracted in the direction Ps, $P's'$, $P''S''$, etc., and being condensed by the objective between x and y cause this part of the scale to be illuminated. There being no possible angle of refraction for light in the prism greater than that for the borderline of total reflection, the part of the scale between x and z remains in shadow.

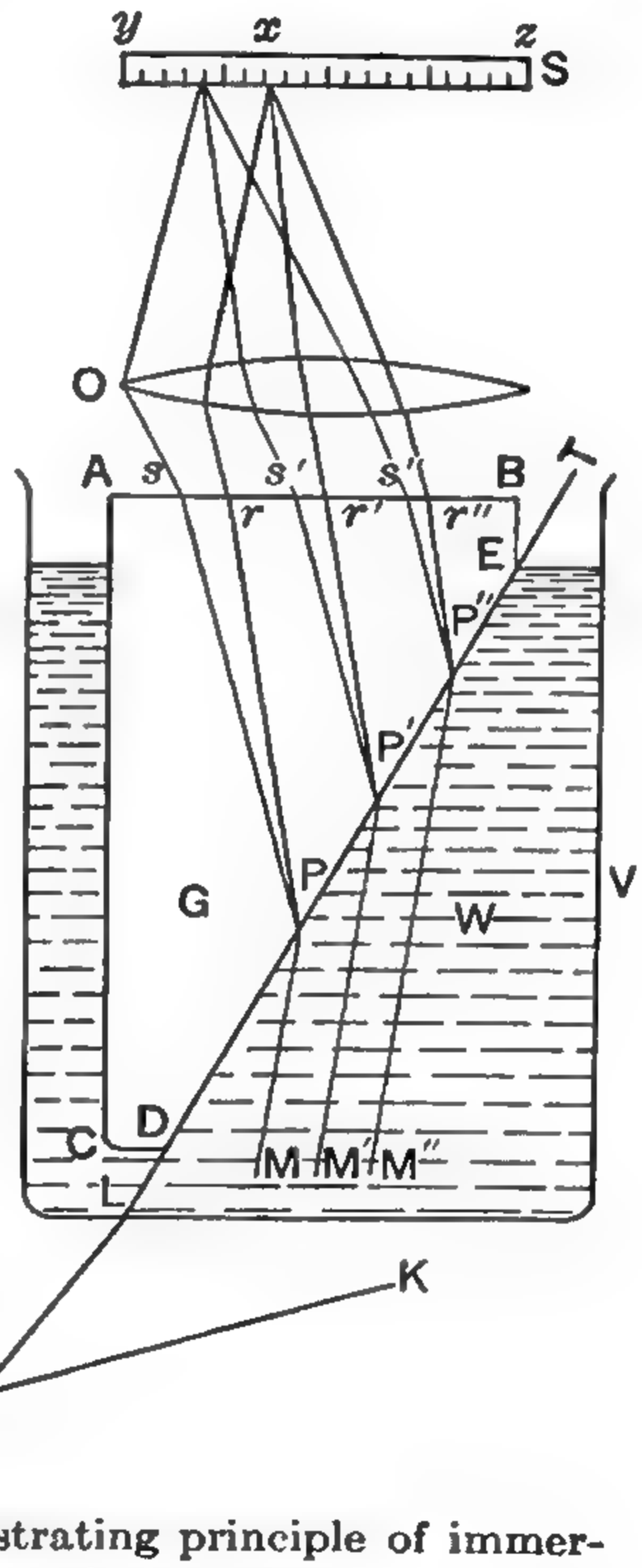
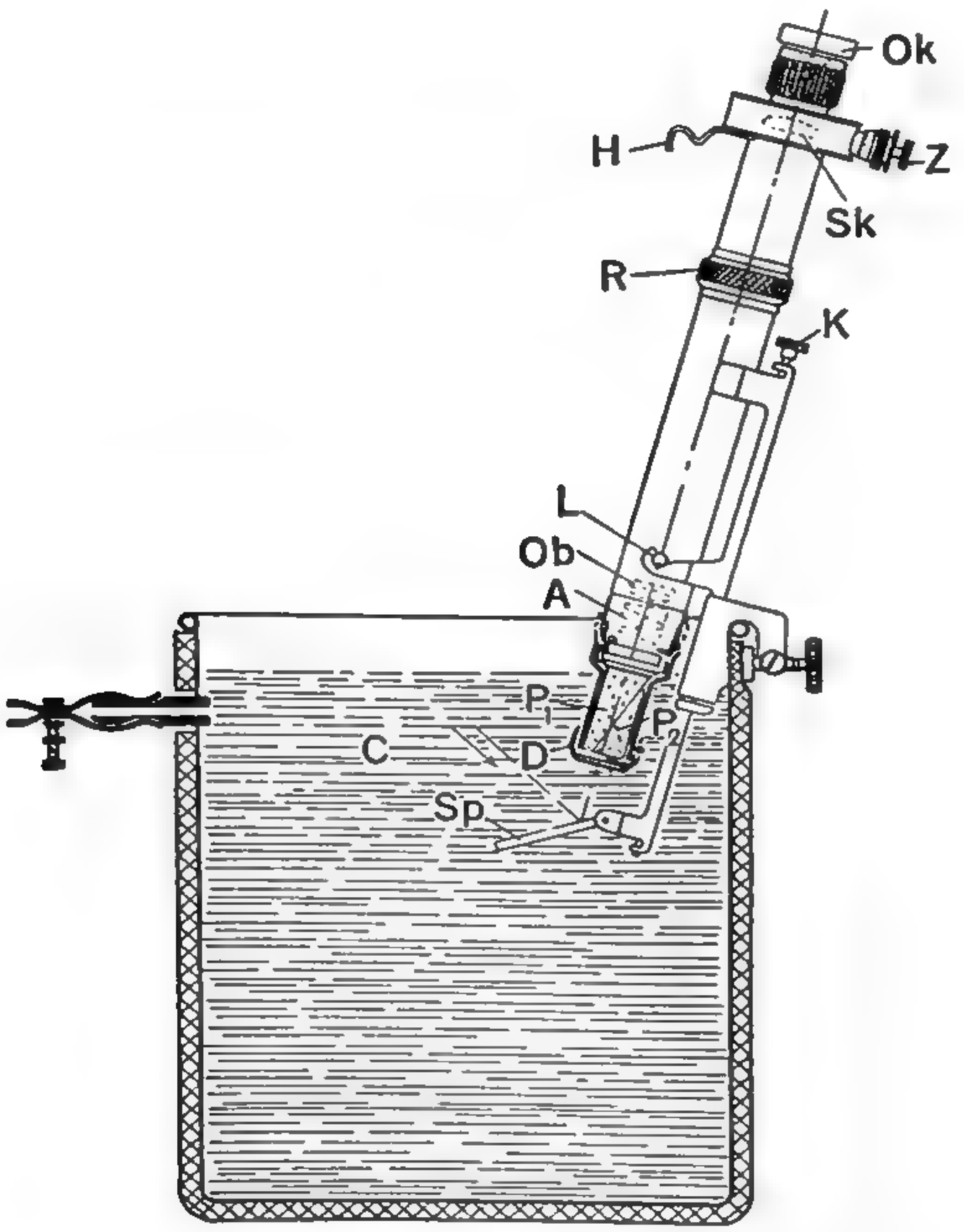


FIG. 54. Illustrating principle of immersion refractometer.

As in the Abbe refractometer, the borderline on account of differences in dispersion is fringed with color and must be corrected by a compensator in the manner described on p. 91. The compensator is placed at *A* (Fig. 55) between the objective *Ob* and the prism P_1 and is rotated by the milled ring *R* until the borderline upon the scale becomes sharp and colorless. The position of the borderline upon the scale marks the reading for the whole division; the fractional division is determined by rotating the micrometer screw *Z*, which controls the scale, until the whole division previously noted is brought into contact



(Courtesy of Carl Zeiss, Inc.)

Fig. 55. Showing construction of immersion refractometer.

with the borderline. The reading of the micrometer drum shows the fractional division which remains to be added. Readings can be made by careful observers to agree within 0.05 to 0.1 scale division, which corresponds to about 2 to 4 units of the fifth decimal place of the refractive index. A special eyepiece is also furnished by Zeiss, which increases the accuracy to 0.02 of a scale division, or about one unit in the fifth decimal place of the refractive index. This is greatly superior to the accuracy in reading the Abbe instrument.

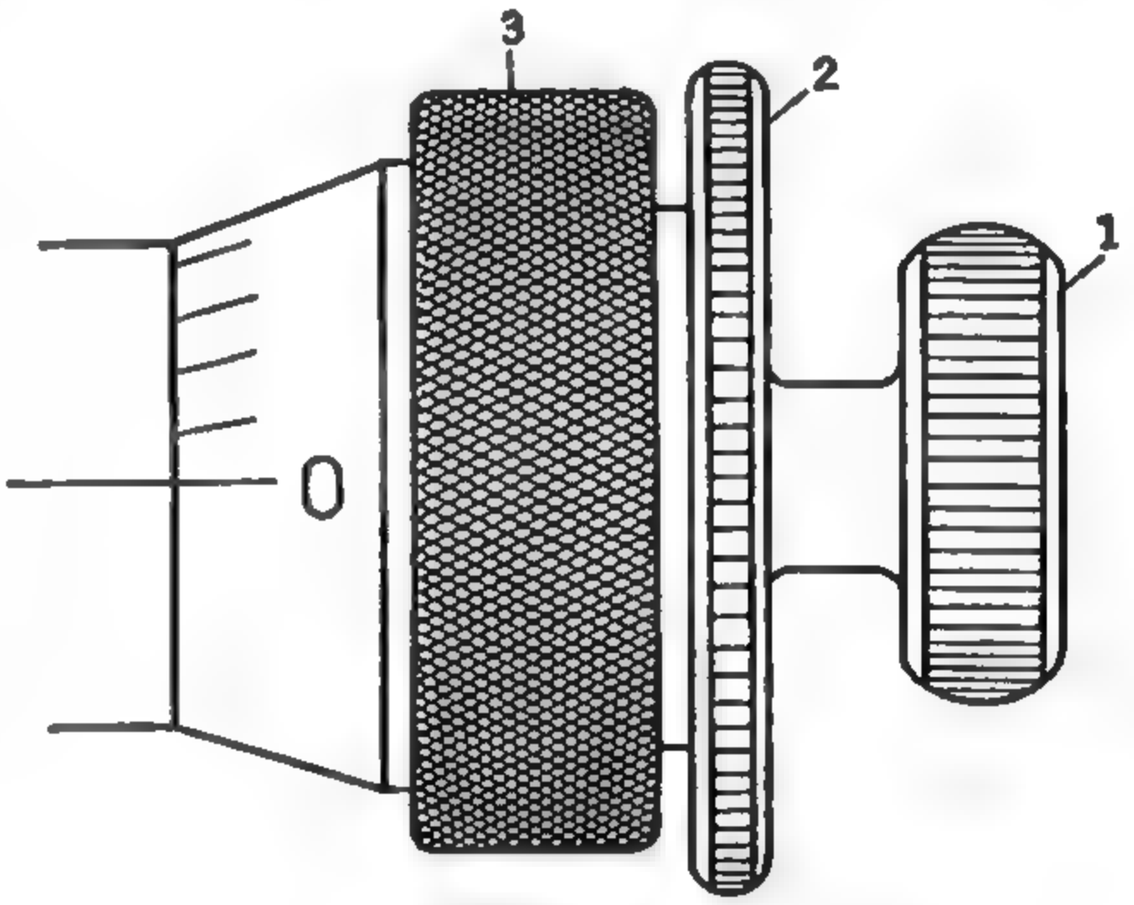
The adjustment of the first prism, comprising the lowest range of the refractive index scale, is made with distilled water, which should give a reading of 15 at 17.5° C. The adjustment, however, can be made at other temperatures according to the following table.

The correctly adjusted refractometer should show for distilled water:

At a temperature of.....	10°C.	11	12	13	14	15	16	17	17.5	18	19°C.
The scale division.....	16.3	16.15	16.0	15.85	15.7	15.5	15.3	15.1	15.0	14.9	14.7

At a temperature of.....	20°C.	21	22	23	24	25	26	27	28	29	30°C
The scale division.....	14.5	14.25	14.0	13.75	13.5	13.25	13.0	12.7	12.4	12.1	11.8

Should the average of several careful readings differ by more than 0.1 division from the reading in the above table for the temperature of testing, the scale should be readjusted. This is done by first setting the micrometer drum (3 in Fig. 56) at 0. The small milled screw 1 is then turned counter-clockwise, to release it. Next, the milled ring 2 is turned until the boundary line coincides exactly with the scale division given by the integer number in the above adjustment table, for the temperature of the water in the beaker. While the milled ring is held tightly in this position the micrometer drum is turned to the decimal fraction in the table. Finally, the micrometer drum and the milled ring are held fast in their position, and the milled screw 1 is tightened again, care being taken that the adjustment is not disturbed. The corrected setting should now be confirmed by a few additional readings.



(Courtesy of Carl Zeiss, Inc.)

FIG. 56. Micrometer screw of the Zeiss immersion refractometer.

The *Z* prism is also adjusted with distilled water, but since its refractive index range is different from that of the first prism the scale reading for water is also different. When correctly adjusted, it should give the readings shown in the following table:

Temperature, °C.	17	18	19	20	21	22	23	24	25
Scale division.....	+0.65	+0.44	+0.22	0.00	−0.23	−0.48	−0.74	−1.00	−1.26

For adjusting the remaining prisms, the manufacturers of the instrument furnish standard solutions or test prisms or plates of fluorite or glass on which the correct scale division or refractive index is inscribed. The standard solutions are used for the calibration in the same way as has been described for the first prism. The adjustment with the test prisms or plates is made by the method of grazing incidence, described on p. 97. The exact procedure used with the Zeiss immersion refractometer has been changed several times since it was first placed on the market, and that for the Bausch and Lomb instrument is different from that for the Zeiss model. The details are therefore omitted here, and the user should follow the directions furnished with each instrument by the maker.

The readings on the scale of the immersion refractometer extend from —5 to +105, and are converted into refractive indices and into percentages of sugar by means of special conversion tables which accompany the instrument. A sugar table for the first prism of the immersion refractometer, up to 21.71 per cent sucrose, has been prepared by Hübener,[42] but it had to be revised on the basis of the new Landt-Schönrock values of the refractive indices of sucrose solutions (p. 102). The refractive indices according to Landt-Schönrock, corresponding to each scale division for the first prism of the Zeiss immersion refractometer, are shown in the Appendix, Table 10. Each tenth of a division of the scale corresponds to about 0.02 to 0.03 per cent sugar, and the concentration can therefore be determined to 0.01 per cent, provided that the temperature is controlled to a corresponding accuracy, that is, within about 0.1° C.

The scale of the Bausch and Lomb immersion refractometers bearing serial numbers 1 to 3999, and again from number 10001 upward, is identical with that of the Zeiss instrument, but on refractometers Nos. 4000 to 10000 it is different, and the conversion tables are not interchangeable. A committee, appointed by the Association of Official Agricultural Chemists in 1931, has recommended[43] that the Zeiss scale be adopted as the standard for the first prism. In order to facilitate conversion of the scale readings on the Bausch and Lomb instruments Nos. 4000 to 10000 into values of the standard scale, the committee has compiled the table shown on p. 121.

The committee has made no recommendations for a standard scale for the higher prisms.

A table showing the percentages of sucrose corresponding to the scale divisions of the *Z* prism, in steps of 0.05 scale division, has been

[42] *Deut. Zuckerind.*, 33, 108 (1908).
[43] *J. Assoc. Official Agr. Chem.*, 16, 85 (1933).

B. & L. Scale, No. 4000 to 10000	n_D	Zeiss Scale	B. & L. Scale, No. 4000 to 10000	n_D	Zeiss Scale
−5	1.32539	−5.0	55	1.34855	55.5
0	1.32737	0.0	60	1.35041	60.5
+5	1.32934	+5.1	65	1.35227	65.6
10	1.33131	10.1	70	1.35411	70.6
15	1.33326	15.2	75	1.35595	75.7
20	1.33521	20.2	80	1.35778	80.8
25	1.33714	25.2	85	1.35959	85.8
30	1.33907	30.3	90	1.36139	90.8
35	1.34098	35.3	95	1.36319	95.9
40	1.34289	40.4	100	1.36497	100.9
45	1.34478	45.4	105	1.36674	106.0
50	1.34667	50.5	...	.....	.....

published by Landt.[44] It also gives the original percentage of sucrose in products the normal weight of which has been diluted to 100 ml.

For controlling the temperature of the water bath, containing the beakers of solution for the immersion refractometer (Fig. 53), the heaters and pressure regulators previously described may be used. If measurements are to be made only occasionally a thermostat bath (Fig. 57), in which the beaker stand is inserted, is sufficient. When the proper temperature has been reached in the beakers the solutions are read in sequence, the refractometer prism being wiped dry after each immersion. When large numbers of solutions are to be tested, each solution as soon as read is replaced by a beaker of fresh solution, thus giving sufficient time for regulation of temperature without interruption of work.

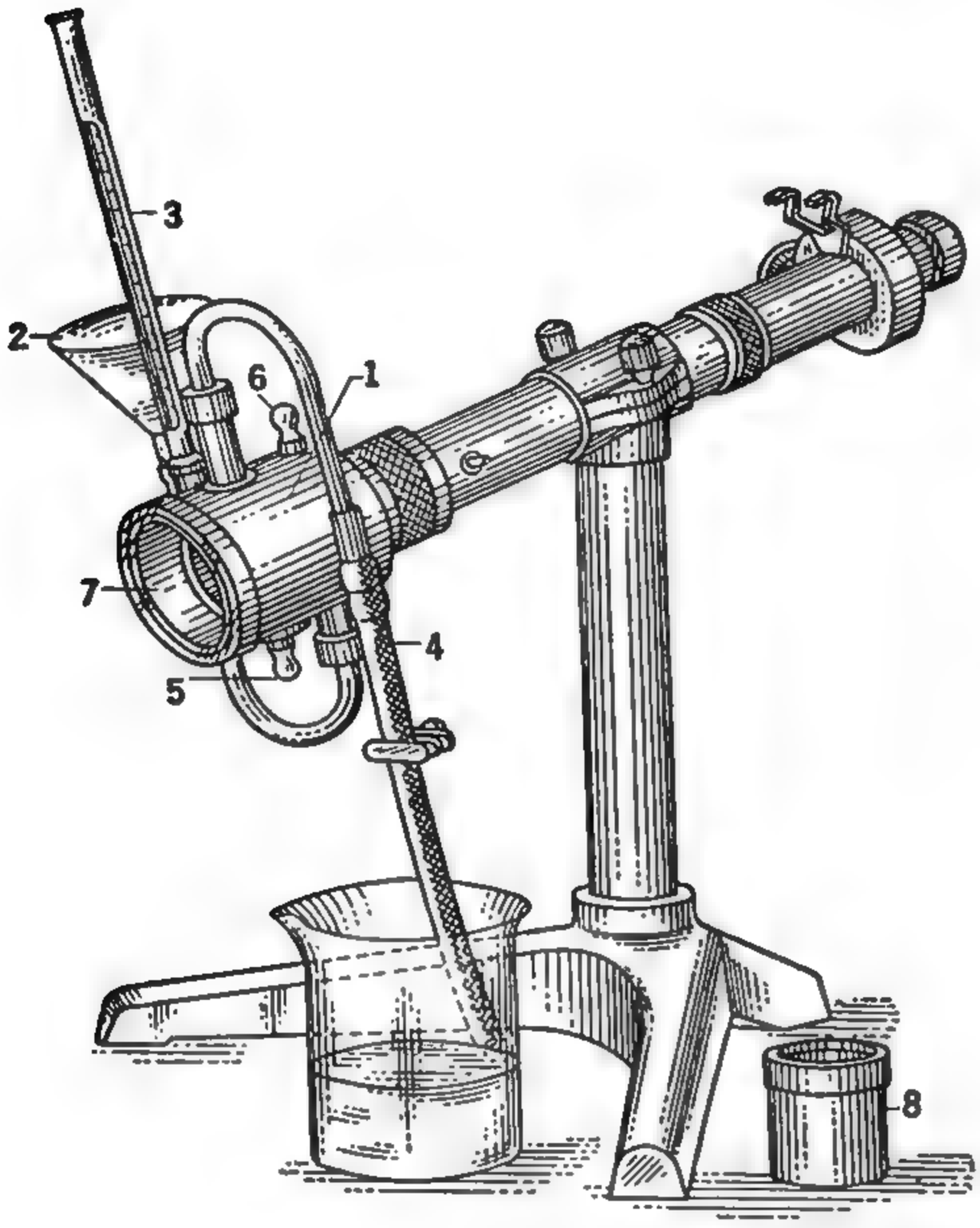
(Courtesy of Carl Zeiss, Inc.)

FIG. 57. Thermostat bath for immersion refractometer.

For work of high precision it is best to use the Höppler thermostat to furnish water of constant temperature.

[44] *Z. Ver. deut. Zucker-Ind.*, 83, 692 (1933).

If sufficient quantities of sample are available, serial measurements of mobile solutions whose refractive index is within the range of one prism may be made in quick succession by means of the Goldbach continuous flow cell, Fig. 58. It is well adapted for routine examination of factory products, variety tests, etc.[45] The cell is slipped tightly over the prism end of the immersion refractometer, which is clamped on an iron support. The sample solution is poured in through funnel 2, and the excess solution runs out through tube 4. After one sample has been read, the solution is displaced by washing twice with the next solution to be examined, and the reading is taken on the third portion. The borderline in the field must be sharp, a blurred line indicating incomplete displacement of the previous solution, or the presence of air bubbles which must be allowed to rise before the reading is taken. The temperature is kept constant by circulating water through the outer jacket by means of the nipples 5 and 6.



(*Courtesy of Carl Zeiss, Inc.*)

FIG. 58. Immersion refractometer with flow-through cell.

When only a few milliliters are available or when the liquids to be examined are very turbid, like raw cane juices, or very dark in color, like molasses, the immersion prism is fitted with an auxiliary prism held in position by means of a metal beaker and cover. The method of use is somewhat similar to that of the Abbe refractometer; the hypotenuse surface of the auxiliary prism is covered with a few drops of solution and then inserted in the beaker against the face of the immersion prism so that a thin layer of liquid is spread between the two.

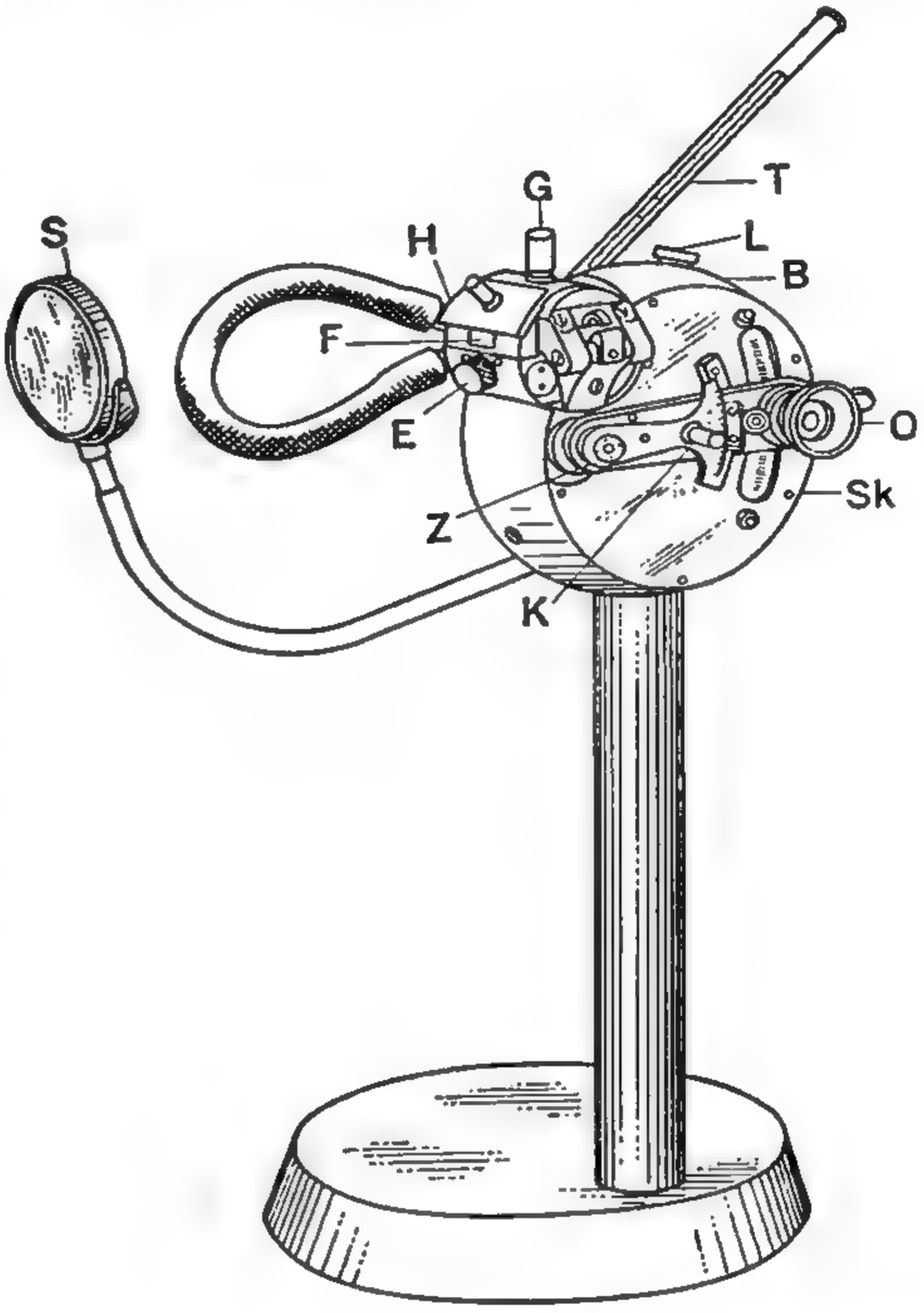
The remarks upon illumination under the Abbe refractometer also apply to the immersion instrument.

[45] See Bachler, *Facts About Sugar*, **28**, 420 (1933).

Special Refractometers for Sugar Work

The Zeiss Sugar Refractometer. This instrument, which is shown in Fig. 59, represents an improvement upon an earlier model designed in 1911 by Löwe and Schönrock.[46] It is especially adapted for use in the sugar industry, but may be applied also to other products, such as oils whose refractive index does not exceed 1.54. The prisms are made of a lighter, less breakable glass, of lower refractive index than in the Abbe instrument.

The apparatus consists of a cylindrical casing mounted upon an upright support and carrying the double prism on the left side of the upper rim. The top prism can be swung back on a hinge so that the surfaces of the two prisms are exposed in one plane, for easy cleaning and recharging. A few drops of the sugar solution are spread with a smooth glass rod on the horizontal surface of the lower prism; the upper prism is then folded shut and clamped in its original position. A beam of light, preferably furnished by a frosted electric lamp, is reflected from the mirror *S* through the upper rectangular opening *F*. The readings are taken through telescope *O*, which can be raised or lowered on a movable arm attached to pivot *Z*. The line of vision being horizontal, the operation of the instrument is more comfortable in routine work than that of the Abbe type.



(*Courtesy of Carl Zeiss, Inc.*)

Fig. 59. Zeiss sugar refractometer.

The refractometer is provided with two scales, both appearing in the field of vision, which is another advantage over the Abbe model. The scale on the left side shows the refractive index, from 1.33 to 1.54,

[46] For a full description of the Löwe-Schönrock refractometer, which is no longer manufactured, see *Z. Ver. deut. Zucker-Ind.*, 64, 10 (1914).

to the third decimal place; the fourth decimal can be estimated by interpolation. The scale on the right-hand side directly indicates percentage of sugar (dry substance) by weight, and is divided into fifths of a per cent from 0 to 50, and into tenths from 50 to 95 per cent.

A reference line is used instead of the cross wires found in other instruments. The line is made to coincide with the borderline in the field of vision, and then the refractive index or per cent sugar is read off where the borderline traverses the scale. Any color fringe appearing in the borderline is eliminated by shifting the handle *K* controlling the compensator, which is a rotating triple prism, similar in construction to that used in the immersion refractometer (*A*, Fig. 55).

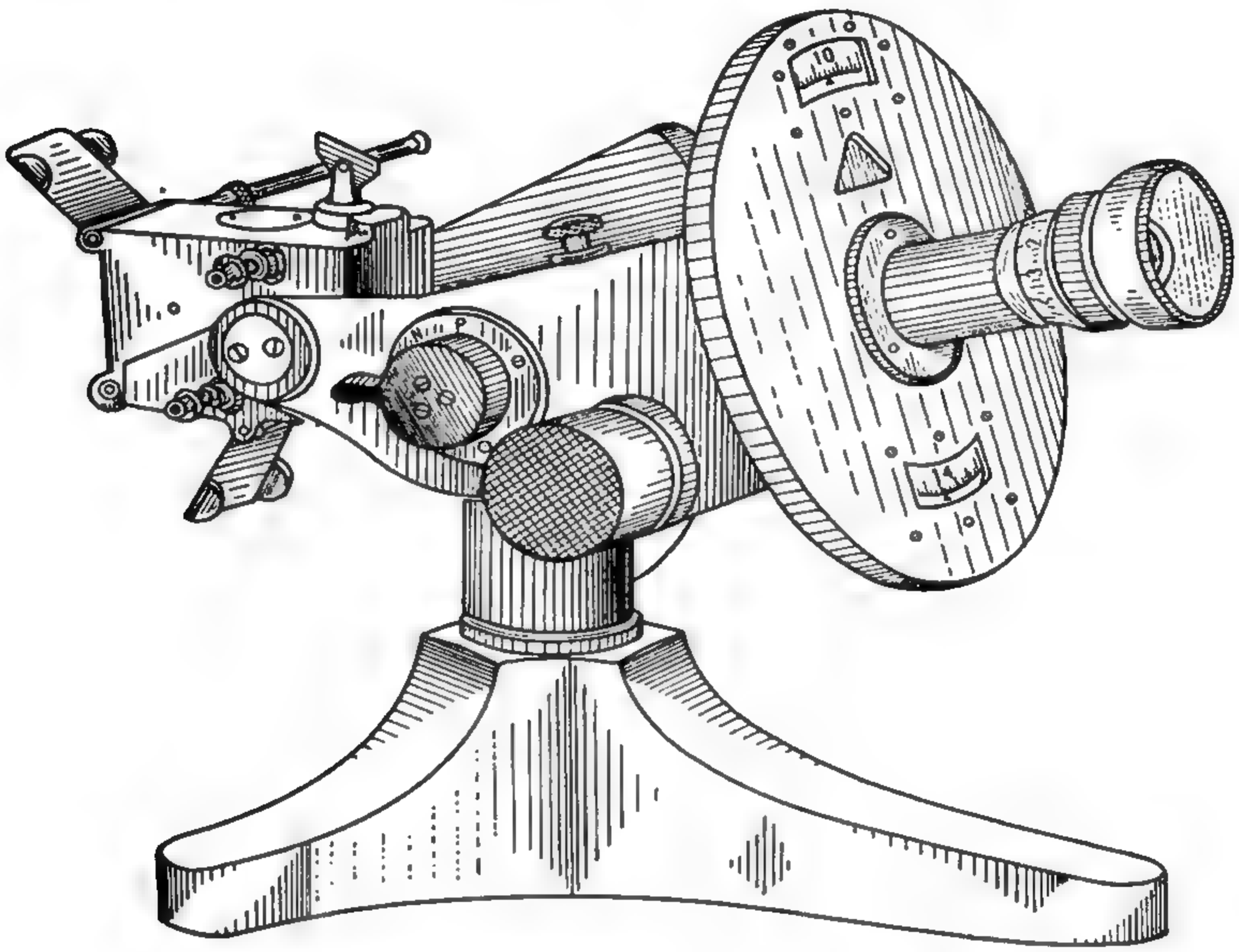
If the product is so dark colored that the boundary line cannot be located by means of transmitted light, it is necessary to resort to reflected light. The rectangular opening *F* is closed with shutter *H*, the stopper is removed from the round hole *E*, and the mirror is turned so that it throws the light directly into *E*. The readings are then taken as explained in the description of the Abbe refractometer, p. 105.

Like the Abbe instrument, this refractometer is made in two patterns, one standardized at 20° C., for use in the temperate zone, and the other at 28° C., for the tropics. If the apparatus is used at a temperature other than that for which it was standardized, a correction must be applied, using Table 7 or 8 in the Appendix.

The correct adjustment of the instrument is verified with distilled water which must read 0 per cent sucrose at the standard temperature. A test plate of known refractive index may also be employed. If the scale is found to be out of adjustment, this may be corrected by unscrewing the small cap *L* on top of the housing, inserting the key furnished with the instrument, and turning the setpin until the boundary line coincides with the correct value of the refractive index of water or of the test plate.

The Goerz Sugar Refractometer. In this instrument, shown in Fig. 60, the telescope is in a fixed position, while the double prism is rotated by means of milled screw heads on both sides. This refractometer has a fixed compensator whose dispersion is equal to the average dispersion of sugar solutions. The apparatus can therefore be used only for sugar products, but not for materials whose dispersion differs materially from that of the compensator. After the solution to be examined has been placed on the prism in the usual way the prism is turned and the boundary line set with the aid of the cross wires, as in the Abbe instrument. The refractive index is then read from the

upper scale, near the periphery of the large disk behind the telescope head, to the third decimal place, the fourth decimal being estimated. The percentage of sugar (dry substance) may be found directly from the lower scale, opposite the other; this scale is divided into halves of 1 per cent, and the tenths can be readily interpolated. The method of adjusting the scale is similar to that used with the instruments already described, the setpin being located on top of the body of the



(Reproduced with permission from Z. Ver. deut. Zucker-Ind., 71, 357.)

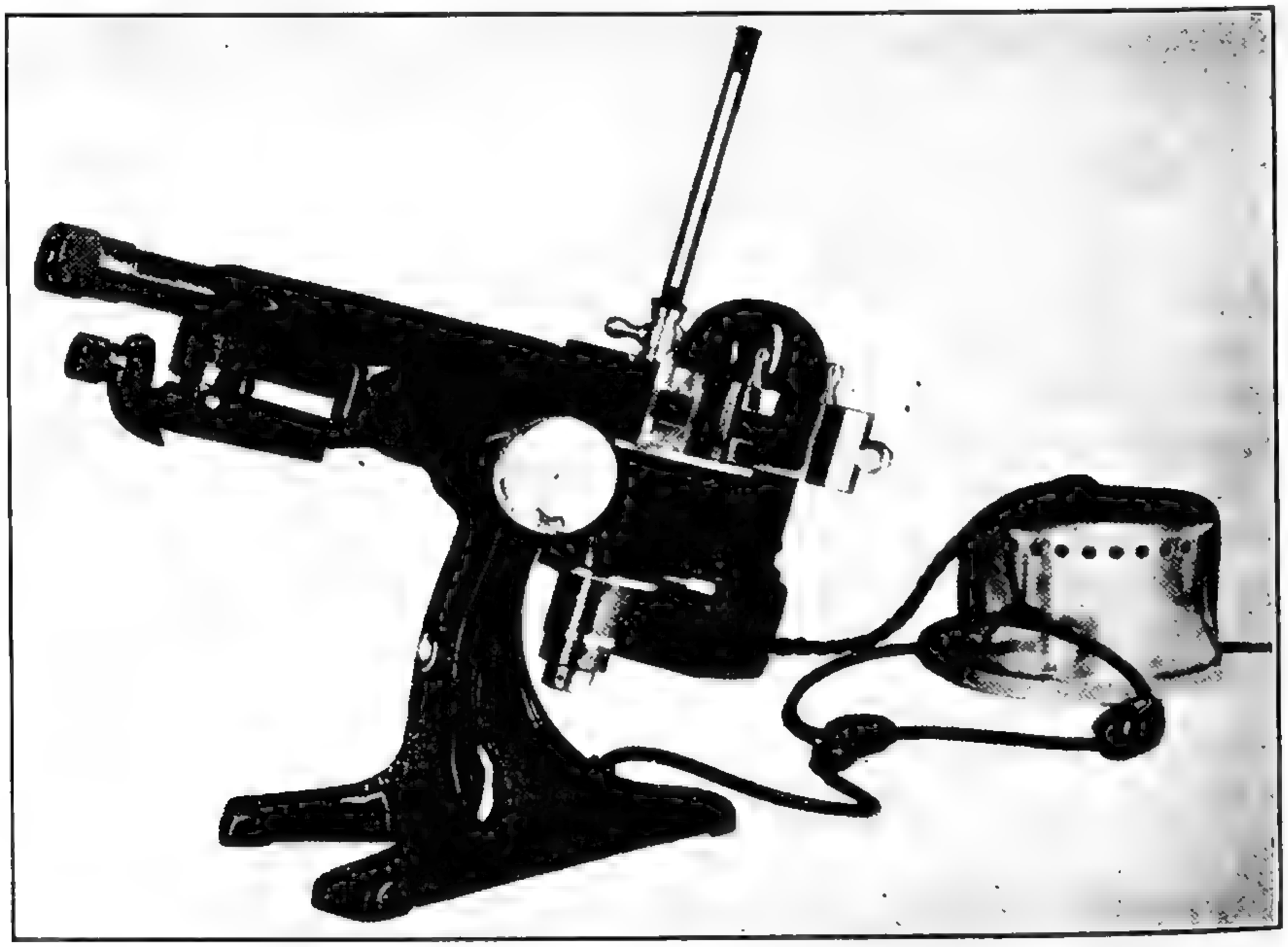
FIG. 60. Goerz sugar refractometer.

instrument, between the scale disk and the prism. This refractometer is also manufactured for standard temperatures of 20° and 28° C.

A sugar refractometer in which both the prism and the telescope are in a fixed position has been designed by Schulz.[47] The boundary line in the field is made to coincide with the reference line by means of a rotating mirror placed between the prism and the telescope. The mirror is actuated by a drum located outside the housing, and the scale is engraved on the periphery of the drum. The instrument is equipped with a sodium-vapor lamp as light source so that no compensator is necessary.

[47] *Z. Ver. deut. Zucker-Ind.*, **87**, 701 (1937).

The Bausch and Lomb Precision Sugar Refractometer.[48] In this instrument, Fig. 61, the Abbe prism is combined with the telescope of the immersion refractometer. This makes it possible to use only a drop or two of sample, the temperature of which adjusts itself quickly to that of the instrument, and at the same time the refractive index can be determined with a precision of about three units in the fifth decimal place over a continuous range from 1.30 to 1.51. The usual



(Courtesy of Bausch & Lomb Optical Co.)

FIG. 61. Bausch & Lomb precision sugar refractometer.

color compensator is dispensed with by employing a sodium-vapor lamp as the light source.

The instrument is mounted on a heavy casting, the optical axis being slightly inclined from the horizontal. The conical bearing about which the prism rotates is thus in a nearly vertical position, and this reduces wear to a minimum. The bearing is 3 inches long and precisely fitted in a casing in order to remove eccentricity and

[48] Forrest, *Proc. Sixth Conference Intern. Soc. Sugar Cane Tech.*, 1938, p. 890. For a full discussion of the optical and constructional features of the instrument, see Straat and Forrest, *J. Optical Soc. Am.*, 29, 240 (1939).

end play. The working parts are arranged so that contamination by spilled liquid or dirt is virtually impossible, and further protection is provided by a semicircular groove at the lower edge of the prism table, with a drain through which the water used for cleaning the prism is removed.

The Abbe prism is of the usual construction but rests on its side. The angle of the prism and the refractive index of the glass used are carefully controlled so that the error in the reading may not exceed three units in the fifth decimal place. The sample is applied to the prism in the usual manner; dilute solutions may be introduced through a funnel-shaped opening while the prism is kept closed. The prism is provided with water jacket and thermometer, as in the Abbe refractometer. The temperature must be controlled within 0.1° C. so as to utilize the maximum precision of the instrument.

The telescope is similar to that of the immersion refractometer, but has no compensator. The scale is mounted on the body of the instrument, and the vernier is attached to the alidade. The scale is evenly divided in arbitrary units and can be read to 12 seconds of arc, which corresponds to 0.00003 unit of the refractive index at $n =$ 1.33, and 0.000015 unit at $n = 1.48$. To take a reading, after the sample has been placed in the prism, the alidade is rotated by turning a small wheel with a handle, at the side of the mounting, until the borderline of total reflection coincides with the center of the cross hairs. The scale is illuminated by a small lamp, lighted by means of a push button at the front of the mounting. The adjustment of the instrument is checked and, if necessary, corrected by means of a test plate of known refractive index, in the same manner as with the Abbe refractometer. The point corresponding to 0 sugar may be checked with distilled water.

The scale readings are converted into refractive indices or per cent sugar by means of tables furnished with the instrument. If the scale were divided to give refractive index or per cent sugar directly, the divisions would be unevenly spaced, and a vernier could not be used. Furthermore, a new scale would have to be engraved for each batch of glass with an index of refraction different from that of the original batch, while the arbitrary scale would only require new tables.

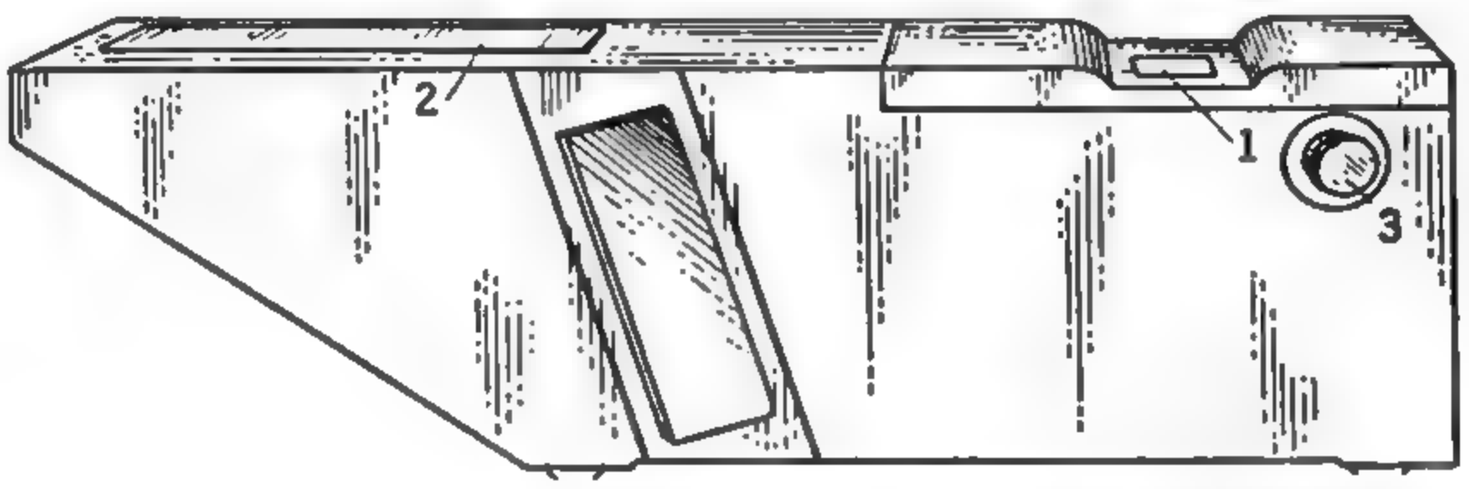
If it is desired to measure dispersion also, a hydrogen tube may be substituted for the sodium-vapor lamp, and readings taken for the C and F lines of the spectrum. The readings are again translated into refractive indices by means of the chart.

A distinctive and useful feature of the instrument is a device that makes it possible to detect the presence of air bubbles in the sample,

which blur the dividing line. A lens is mounted within the telescope, by which the rear face of the refracting prism may be observed directly, and the air bubbles readily discovered. The lens can be rapidly inserted or withdrawn by means of a small lever.

The instrument can also be obtained equipped with a prism of higher refractive index, covering the range from $n = 1.40$ to $n = 1.71$, for measuring oils and solids of high refractive index. The two prisms, however, are not interchangeable, as in the immersion refractometer.

The Projection Refractometer. This instrument, manufactured by Bellingham and Stanley, and shown in Fig. 62, may be used for rapid measurements of refractive index or per cent sugar at room temperature, in routine work where high accuracy is not required. It



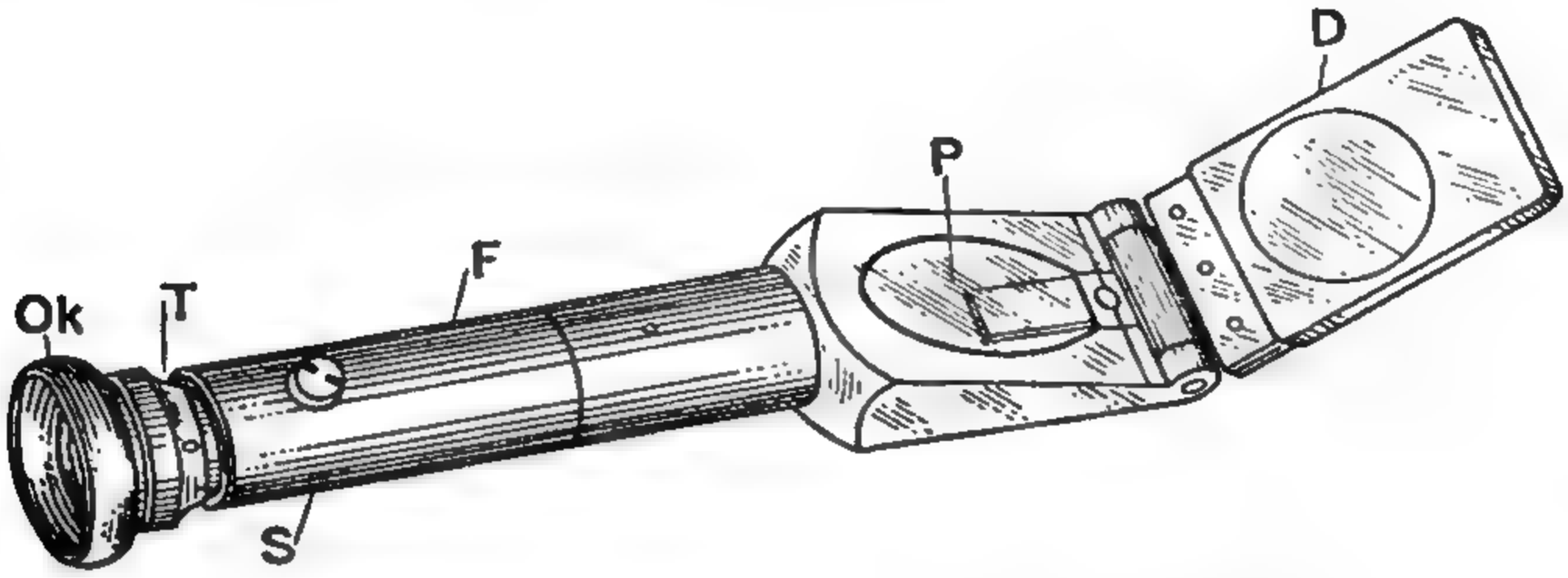
(Courtesy of Bellingham and Stanley.)

FIG. 62. Bellingham and Stanley projection refractometer.

has a single prism, 1, illuminated by reflected light from a lamp mounted inside of the housing. The scale is read, at 2, with the naked eye. There is a double scale; on one side it reads from 30 to 90 per cent sugar, tenths of 1 per cent being estimated, and on the other side it shows refractive indices, from n_D 1.380 to 1.450 in steps of 0.002, and from 1.450 to 1.517 in steps of 0.001. The boundary line is set by milled head 3. On the rear of the housing, opposite 3, is another milled head by means of which either a diffusing screen or a yellow filter may be interposed in the illuminating beam in order to increase the sharpness of the boundary line. Heavy sirups, pastes, etc., are placed directly on the prism, and the reading is taken at once. An auxiliary prism which fits over the measuring prism is used for testing volatile liquids or dilute solutions.

The Zeiss Hand Sugar Refractometer. This apparatus, Fig. 63, has been designed for quick determinations of the approximate total solid content of sugar products, and especially for the testing of beets and canes in the field. It consists of one straight tube, carrying a fixed prism with hinged cover at one end, and the telescope at the other. There is no compensator because experience has shown that under the experimental conditions there is practically no color fringe.

However, when readings are taken a green light filter is placed over the eyepiece, because this gives the sharpest contrast between the more shaded and the less shaded portions of the field. The scale reads in per cent sugar, in whole units from 0 to 10 per cent, and in half units from 10 to 30 per cent. Its range is thus limited to rather dilute solutions, such as beet and cane juices.

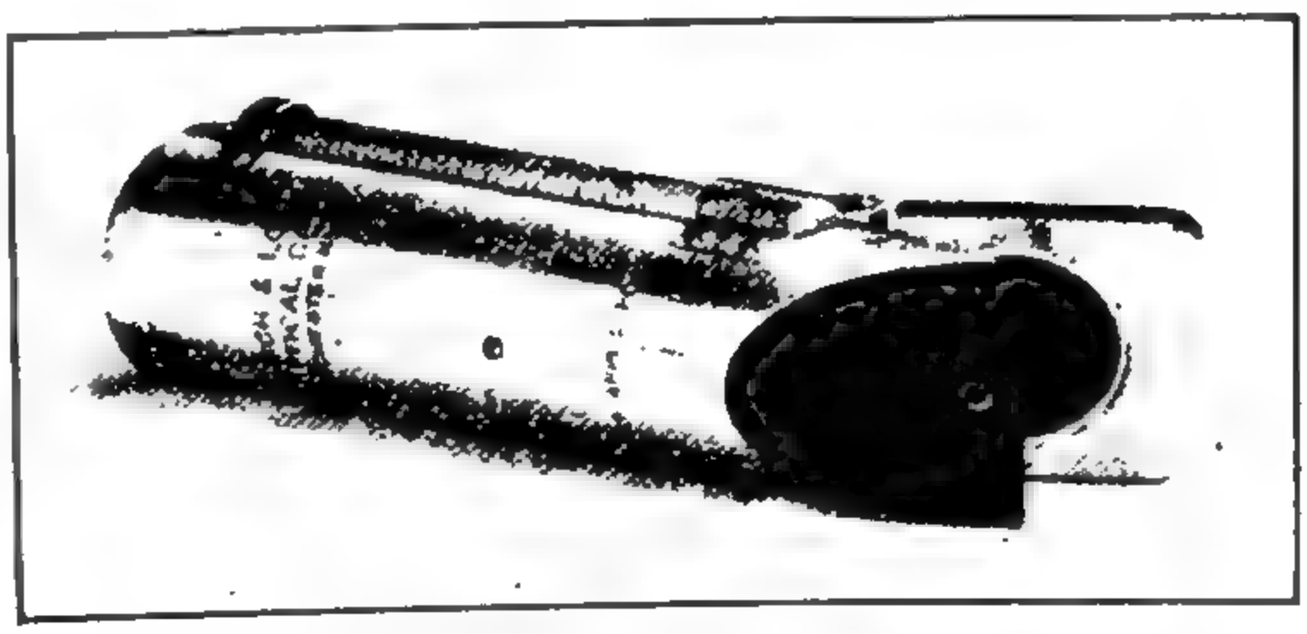


(Courtesy of Carl Zeiss, Inc.)

FIG. 63. Zeiss hand sugar refractometer.

In using the instrument the cover *D* is folded back, the solution applied to the prism surface, and the cover closed again. The instrument is then held horizontally towards the sky or other light source. The telescope is focused on the scale, and the per cent sugar is read off where the boundary line intersects the scale. The 0 point of the scale must be checked with distilled or drinking water of the temperature at which the instrument is to be used. If the correction is small, it may be applied to each reading; otherwise the scale may be adjusted by turning the adjusting screw *S* with the key furnished with the apparatus.

A similar, small hand refractometer which can be slipped into the pocket is manufactured by Bellingham and Stanley.



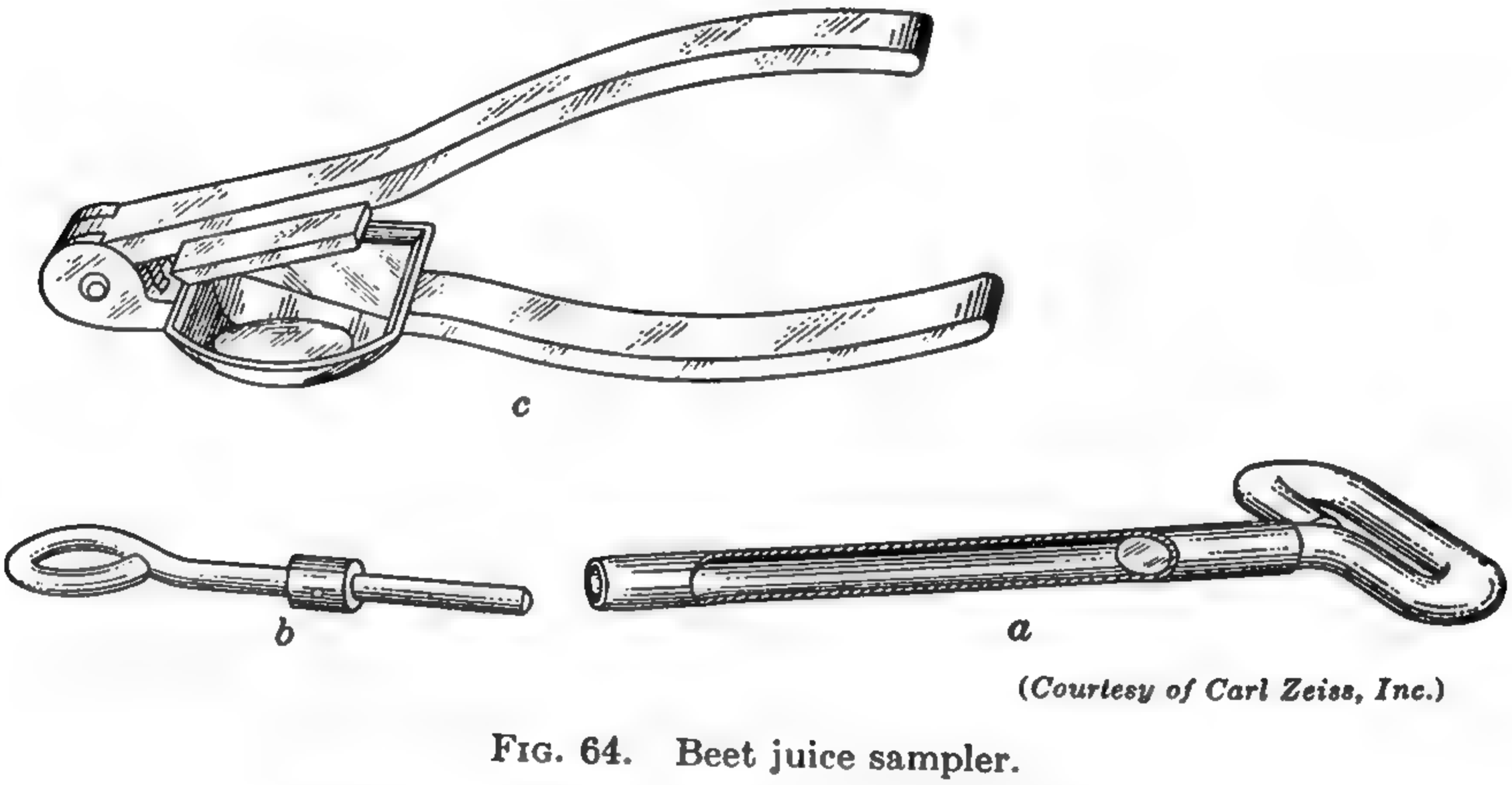
(Courtesy of Bausch & Lomb Optical Co.)

FIG. 63*a*. Bausch & Lomb hand sugar refractometer.

The Bausch and Lomb Hand Refractometer. This refractometer, Fig. 63*a*, is also small enough to be carried in the pocket. It has a sugar scale divided to 0.5 per cent sugar. Two models are available, one reading from 0 to 60 per cent, the other from 40 to 85 per cent. The instrument is calibrated at 20° C., and is provided with

a thermometer indicating directly the corrections to be added (black scale) or subtracted (red scale) at the prevailing temperature.

Various tools have been devised for collecting juice samples from beets or cane in the field. In the beet industry[49] the corer *a*, Fig. 64, is extensively used to remove a small cylindrical sample from the beet.

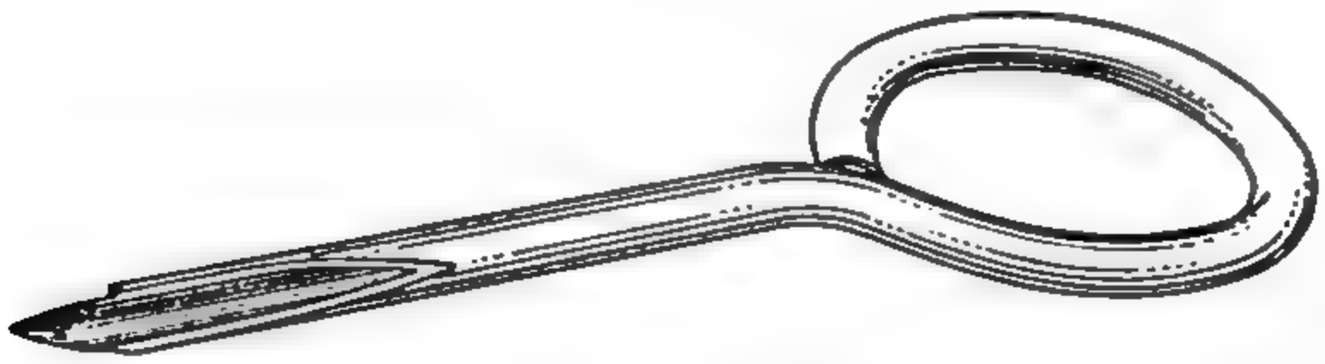


(Courtesy of Carl Zeiss, Inc.)

FIG. 64. Beet juice sampler.

a, Beet corer; *b*, Rod for removing core; *c*, Hand press.

The core is pushed out with the rod *b*. Six such cores, from as many different beets, are placed in the hand press *c*, which is then tightly closed. The expressed juice runs into a small depression, where it is mixed with a glass rod, and then transferred to the refractometer prism.



(Courtesy of Carl Zeiss, Inc.)

FIG. 65. Gembol knife for sampling juice of standing sugar cane.

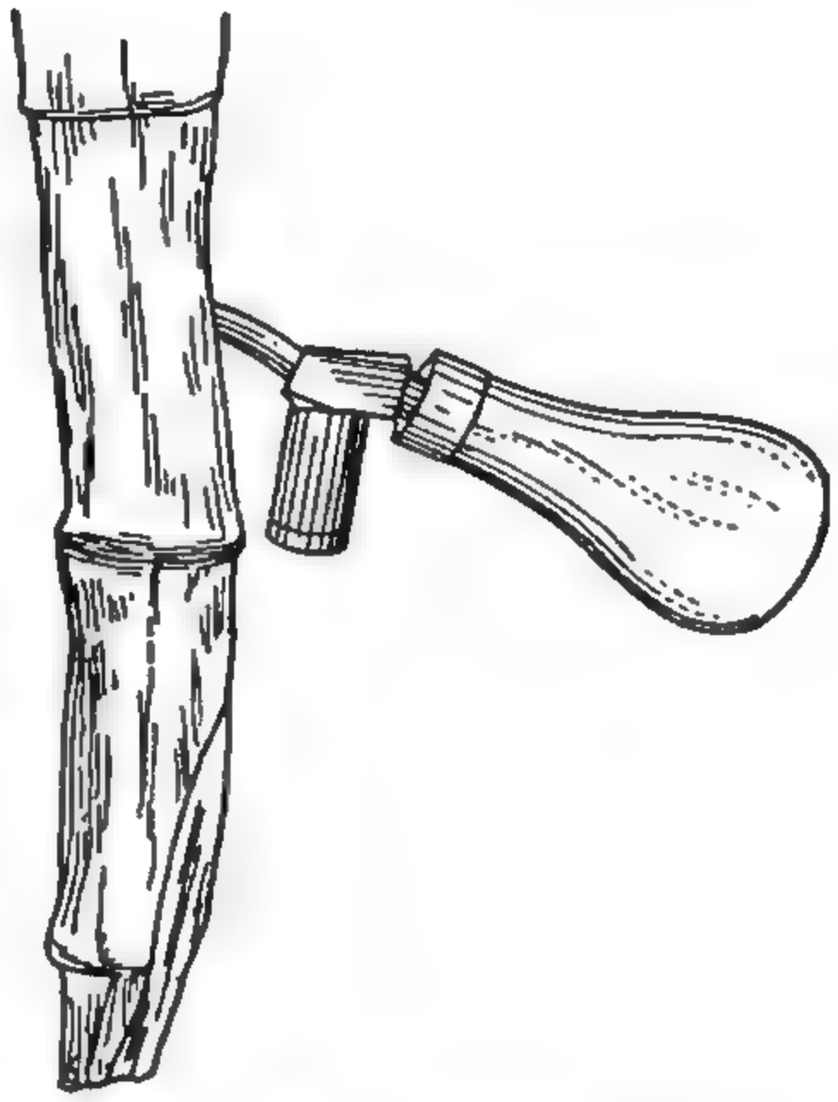
In Java the so-called "Gembol" knife,[50] Fig. 65, is employed to withdraw juice samples from standing cane. The point of the curved knife is pushed horizontally into the stalk, up to the collar, and slightly twisted. The pressure exerted extracts a drop of juice which is poured on the refractometer prism. A composite sample of juice may be collected from a number of cane stalks by means of a punch described by

[49] Hering, *Z. Ver. deut. Zucker-Ind.*, **78**, 345 (1928); Dreyspring and Krügel, *Superphosphat* 1929, No. 8.

[50] Levert, van der Woude, and van Dillewijn, *Arch. Suikerind.*, **40**, II, 629 (1932).

Crosby[51] and shown in Fig. 66. It is similar to the Gembol knife, but has a small reservoir.

The wounds caused in standing cane by the methods just described do not materially affect the further development of the plant. Damage to the growing stalk may be further reduced by the use of a hypodermic syringe, as suggested by Khanna.[52] A spring is inserted under the plunger of the syringe. The plunger is pressed down and the needle inserted through the rind of the cane. When the plunger is released a few droplets of juice are removed from the cane, and these are pressed out again upon the refractometer prism.



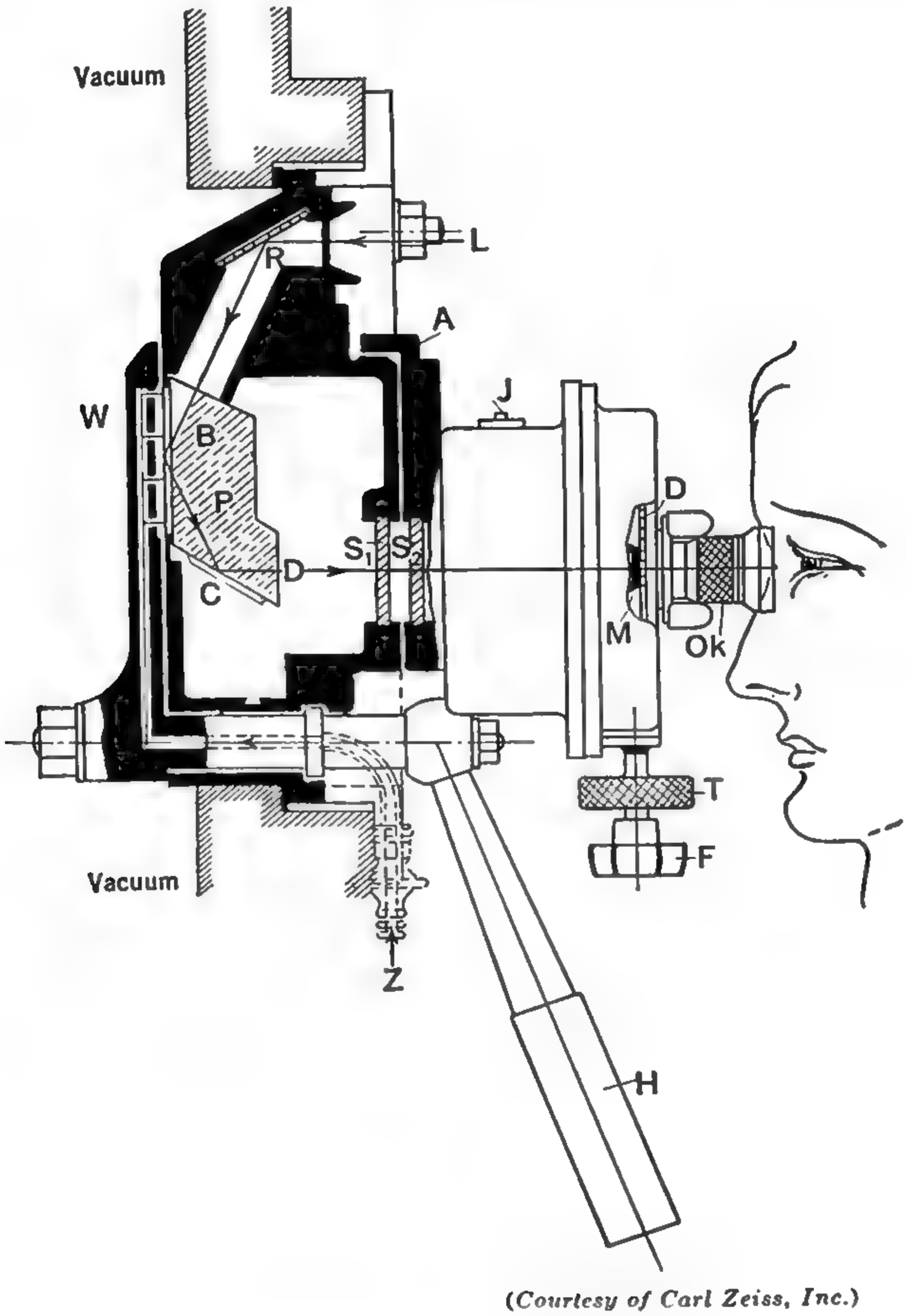
(Reproduced with permission from Repts. Assoc. Hawaiian Sugar Tech., 15, 11.)

FIG. 66. Crosby's sugar cane sampler.

The Pan Refractometer. This instrument is designed for use exclusively in the factory. It is permanently mounted into the wall of the vacuum pan, evaporator, or pipe line, etc. Its use to control sugar boiling in the vacuum pan is based on the fact that crystals present in a mother liquor do not affect the refractive index of the latter because there is usually a film of liquid between the crystals and the prism. The principle of the factory refractometer may be understood from Fig. 67. The light L from a 50-cp. electric lamp outside the pan is reflected at R, again reflected at the rear surface of the prism which is in contact with the massecuite, and is then reflected once more at C into the telescope Ok, through the protecting glasses S_1 and S_2. As with the hand refractometer, no compensator is necessary, but a green light filter is provided. The temperature corrections for the interval from 60° to 100° C., and for concentrations varying from 70 to 95 per cent sugar (dry substance), have been carefully determined. On the basis of the results obtained, series of correction curves were drawn which are built into the instrument in the form of a diapositive, and which are moved up and down through the field of vision by means of screw T. Handle H actuates a wiper which covers the prism surface when the refractometer is not in use. To take a reading, the prism surface is exposed by turning H to the right. Then screw T is turned until the mark is at the beginning of the temperature scale (60° C.), so that the curves are outside the field of

[51] *Reports Assoc. Hawaiian Sugar Tech.*, 15, 11 (1936).
[52] *Indian J. Agr. Sci.*, 8, 719 (1938).

vision. Then the telescope is focused, and the boundary line is set to the center of the reference circle by turning screw *F*. Next the temperature of the massecuite is read on the thermometer, and *T* is set to the temperature observed. Now the percentage of sugar (dry



(Courtesy of Carl Zeiss, Inc.)

FIG. 67. Pan refractometer.

substance) may be read through the telescope where the corresponding concentration line passes through the center of the circle mark. The scale is divided into units of per cent sugar, and the tenths can be estimated. When the massecuite is becoming highly concentrated, toward the end of the boiling period, it sometimes happens that crystals

form directly on the surface of the prism. In that case a small quantity of hot water is sucked up through nipple *Z*. The water quickly rises to *W* and dissolves the crystals. Cold water should not be used because it is likely to crack the prism. The water rapidly dissipates into the pan through circulation, and a reading can be taken shortly after. The adjustment of the scale must be verified by means of a glass plate of known refractive index before the instrument is permanently mounted. The setpin for adjusting the scale is located under cover *J*. The telescope part of the instrument is detachable, and one is sufficient for use with a number of refractometers mounted on different vacuum pans or other apparatus.

In his choice of a refractometer the analyst must be guided by requirements. For general laboratory work, involving the examination of other products besides those of a saccharine nature, and including substances of high refractive index, the Abbe instrument is the most useful. If the anticipated maximum index of refraction does not exceed 1.54, then the Zeiss sugar refractometer is as universally applicable as the Abbe and is much more rapid in its operation. For sugar work exclusively the Goerz sugar refractometer fully answers the requirements. Where greater accuracy is required than is attainable with the instruments named, the immersion refractometer should be used, but it requires also more careful temperature control. The greatest possible accuracy is offered by the Bausch and Lomb precision refractometer.

Reliability of the Various Methods for Determining Total Solids

A comparison of the results obtained by three or more different methods upon the same sample or lot of samples is shown in Table XXIX. Column *a* gives the known solids in some sirups, calculated from the concentrations of the constituents used; column *b*, the solids determined by drying on sand at about 100° C.; column *c*, the solids found by drying on quartz sand or asbestos in vacuo at 70° C.; column *d*, the Brix based on the specific gravity of the undiluted sample; column *e*, the Brix found by dilution with an equal weight of water; column *f*, the refractometer solids, expressed as sucrose; and column *g*, the solids obtained by the distillation method with toluene as the boiling liquid.

The highest figure is usually obtained with the Brix spindle on the diluted sample, because of contraction. The only exceptions are two sirups high in invert sugar, where the contraction is more than

compensated for by the fact that invert-sugar solutions have a lower density than sucrose solutions of the same concentration. No general rules can be given for the solids obtained by other methods, the results depending entirely on the composition of the products. With beet molasses the dry substance is generally somewhat higher than the Brix of the undiluted sample, and higher also than the refractometer solids. But for beet refinery molasses Mikolášek found the opposite

TABLE XXIX

Comparison of Results for Total Solids of Sugar Products Obtained by Different Methods

Product	a	b	c	d	e	f	g
Beet molasses,[53] 25 samples	.....	79.75	.....	78.85	.....	78.50	.....
Beet molasses,[54] 1 sample	.....	79.59	80.08	.....	81.10	78.82	.....
Beet molasses,[55] 1 sample	.....	78.70	79.16	.....	84.61	78.31	.....
Beet molasses,[56] 1 sample	.....	76.6	.....	75.4	.....	.....	76.6
Beet molasses,[56] 1 sample	.....	78.4	.....	76.5	.....	.....	78.7
Beet refinery molasses,[57] 4 campaigns	.....	78.89	.....	79.35	.....	79.40	.....
Cane molasses,[54] 1 sample	.....	74.81	75.63	.....	80.77	75.96	.....
Cane molasses,[55] 1 sample	.....	74.29	75.78	.....	79.71	75.13	.....
Cuban blackstraps,[58] 7 samples	.....	.....	76.39	.....	.....	78.72	74.12
Cane molasses,[59] 1 sample	.....	73.3	.....	.....	77.3	75.3	.....
Refinery blackstraps,[58] 4 samples	.....	.....	78.50	.....	.....	79.85	77.57
Unfiltered refinery sirup[58]	.....	.....	77.39	.....	.....	77.0	74.00
Synthetic sirup,[54] 25% invert sugar	66.90	66.80	67.11	.....	67.43	65.67	.....
Synthetic sirup,[55] 22% invert sugar	69.62	69.64	70.26	.....	69.95	69.37	.....
Inverted sucrose sirup[60]	80.69	.....	.....	.....	80.14	78.85	.....
Inverted sucrose sirup[60]	.....	.....	79.09	78.35	79.3	78.2	.....

to be true. With raw cane molasses the refractometer solids are usually higher than the dry substance, but in one instance a lower value was found than by drying in vacuo at 70° C. Refractometer solids may be higher or lower than the dry substance, depending largely on the relative quantities of invert sugar which lower the results obtained, and of salts which may increase or decrease them. In the synthetic sirups the results obtained by drying check fairly well with the known solids, but the individual results of different observers

[53] Šandera, *Z. Zuckerind. čechoslovak. Rep.*, **53**, 1 (1928/29).
[54] Brewster, *J. Assoc. Official Agr. Chem.*, **7**, 354 (1923/24).
[55] Brewster, *J. Assoc. Official Agr. Chem.*, **8**, 375 (1924/25).
[56] Výskočil, *Z. Zuckerind. čechoslovak. Rep.*, **50**, 289 (1925/26).
[57] Mikolášek, *Z. Zuckerind. čechoslovak. Rep.*, **52**, 9 (1927/28).
[58] Markovits, *Louisiana Planter*, **76**, 90 (1926).
[59] Whaley, *Mundo Azucacero* **4**, 345 (1916/17).
[60] Schneller, *J. Assoc. Official Agr. Chem.*, **9**, 156 (1926).

showed large variations. The distillation method checks fairly well with the dry substance of beet molasses, but tends to give low results on cane products because of destruction of reducing sugars at the high temperature employed.

Theoretically the drying method should yield correct results, but in practice it has been found that even at low temperatures the results are considerably affected by the ratio of sand or asbestos to dry matter, and it is often found impossible to reach constant weight even after prolonged heating. The specific gravity and the refractive index can be determined with much greater precision, but the results differ more or less from the true dry substance, depending on the nature and quantity of the non-sucrose constituents.

Davies[61] and others have made attempts to correlate the results obtained by different methods through equations or through simple factors. Though such correlations are useful for single factories, the constants in the equations found in one place are not of general application.

Physical constants other than specific gravity or refractive index may in certain cases be used for determining the concentration of sugar products. Chataway[62] proposed the measurement of viscosity for this purpose in the examination of honey. The method has been improved by Oppen and Schuette,[63] who give an empirical equation and a convenient graph for finding the moisture content of honey from the relative viscosity measured with a falling-sphere viscosimeter (see p. 502). This apparatus was calibrated with honeys the moisture in which had been determined by the official vacuum drying method of the Association of Official Agricultural Chemists. The viscosity method gave the moisture content of 29 honey samples of different floral types with an average error of 0.2 per cent and a maximum error of 0.7 per cent. It should be used with caution, however, because the honey dextrins undoubtedly affect the results.

[61] *Intern. Sugar J.*, 34, 402 (1932).
[62] *Can. J. Research*, 6, 532 (1932).
[63] *Ind. Eng. Chem., Anal. Ed.*, 11, 131 (1939).

CHAPTER V

POLARIZED LIGHT; THEORY AND DESCRIPTION OF POLARIMETERS

In order to arrive at a sufficiently clear understanding of the optical principles which underlie the construction and manipulation of polariscopes, a brief reference must be made to the physical theories of light.

According to the undulatory theory of Huygens, light consists of vibrations or wave motions of the luminiferous ether, the imponderable medium which pervades all space and penetrates all matter.

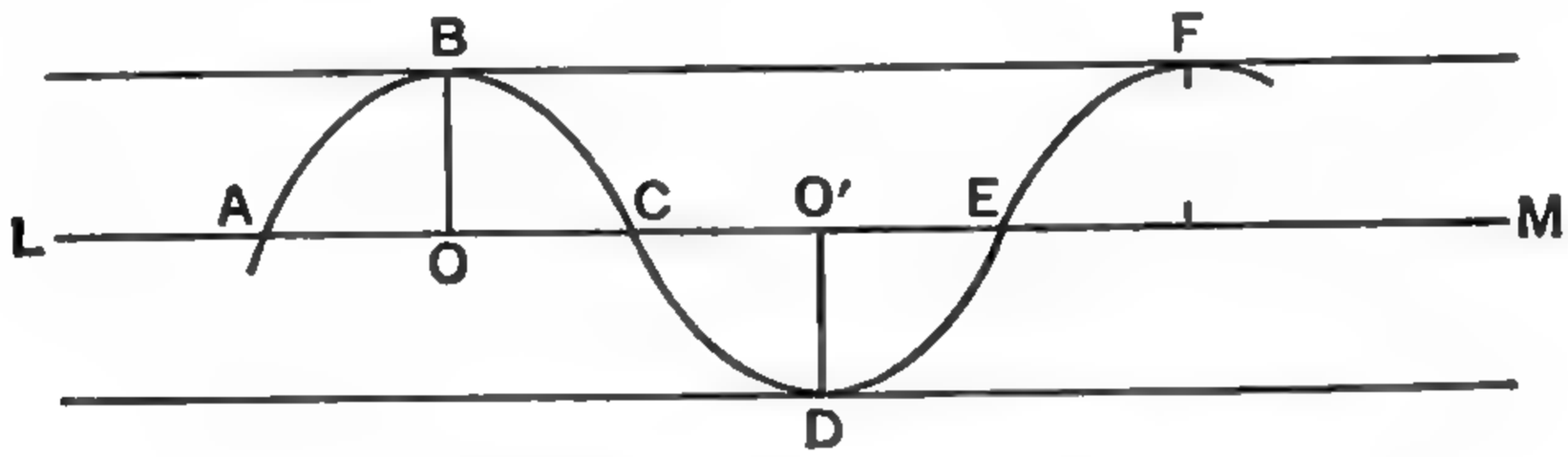


FIG. 68. Illustrating principle of a light wave.

Waves of light, contrary to those of sound, vibrate transversally instead of longitudinally. In Fig. 68 a graphic representation is given of a light wave vibrating transversally to the direction of motion *LM*. The plane of vibration of ordinary light takes all possible positions about this line of motion. The distance *OB* or *O'D* from the middle to the extremity of an oscillation is known as the amplitude of the wave. The distance from *A* to *E* (points in the same phase) is known as the wavelength (λ), which for light is expressed in millionths of a millimeter ($m\mu$), or in Ångström units $Å = 0.1\ m\mu$). The number of waves per second is called the rate of vibration or frequency (*N*). If the velocity of light through a homogeneous medium is *V*, then $N = V/\lambda$.

According to Maxwell's electromagnetic theory, which was later confirmed by the work of Hertz, there are two sets of transverse vibrations in the transmission of a ray of light, the one an electric displacement of the ether, and the other a magnetic displacement, the planes of these being perpendicular to each other. Since the introduction of the quantum theory our conception of the nature of light

has been materially modified, but for the purposes of this discussion the simple wave theory is perfectly satisfactory.

The intensity of a ray of light is proportional to the square of the amplitude; the color depends upon the rate of vibration of the ether wave. The color of light may, therefore, be expressed mathematically in terms of the rate of vibration N or of its wavelength λ. The values of N and λ for the average ray in each color of the spectrum are given in Table XXX.

TABLE XXX

Color	Rate of Vibration per Second (N)	Wavelength (λ) in Millionths of a Millimeter ($m\mu$)
	Billions	
Red	437	683
Orange	485	615
Yellow	534	559
Green	582	512
Blue	631	473
Indigo	679	439
Violet	728	410

The human eye is sensitive to light of vibration periods between about 366 and 804 billion per second, and of wavelengths between about 820 and 373 $m\mu$. Ether waves of greater length than 820 $m\mu$ constitute the so-called infra-red or heat rays, and those of shorter length than 373 $m\mu$ the so-called ultra-violet or chemical rays.

Light of definite wavelength is exceedingly important in making polariscopic measurements, and this is generally secured by using incandescent vapors of certain metals, as sodium, mercury, etc., or of metallic salts, which give bright spectral lines whose wavelengths are absolutely defined. The prominent lines of the different elements are usually designated by the letters of the alphabet, which have been adopted to mark their positions in the solar spectrum. For the sodium line[1] D, to which nearly all polariscopic measurements are referred, $\lambda = 589.3$ $m\mu$.

The vibrations of ordinary light proceed in an infinite number of planes. By means of various special contrivances it is possible, however, to affect a beam of light so that the electric and magnetic vibrations will each proceed in a single plane. Such light is said to be plane polarized; the plane to which the electric vibration of the waves is perpendicular is called the plane of polarization.

[1] The sodium line is double; the component D_1 has a wavelength of 589.6 $m\mu$ and the brighter component D_2 a wavelength of 589.0 $m\mu$. The average wavelength of the two lines, 589.3 $m\mu$ (more exactly 589.25 $m\mu$), is the value taken for D.

The polarization of light was first noticed by Huygens in 1678, while studying the refraction of light in a crystal of Iceland spar. No satisfactory explanation of the phenomenon was made, however, until Malus, in 1808, discovered that the polarization noticed by Huygens in Iceland spar could also be produced by reflection.

Polarization by Reflection. If a beam of light (as *LO* in Fig. 43) falls upon the smooth surface of a transparent substance, it is decomposed into reflected and refracted rays. The reflected rays at a definite angle of incidence are completely polarized, the plane of the lines of incidence and reflection being the plane of polarization.[2] These observations, according to Fresnel and Arago, could be explained only by supposing that the vibrations in a light wave are transverse to the direction of motion, and that during reflection these vibrations are reduced to a single plane, which is perpendicular to the plane of polarization.

The angle of incidence at which reflected light is completely polarized is called the polarizing angle, and varies according to the refractive power of the reflecting substance. This relationship is expressed by Brewster's law, viz.: The tangent of the polarizing angle is equal to the index of refraction for the reflecting substance, or $\tan i = n$. The polarizing angle of glass ($n = 1.54$) is accordingly about 57°.

The Nörremberg Apparatus. A simple apparatus for producing and studying polarized light is that of Nörremberg, shown in Fig. 69. *A* and *B* are two mirrors of black glass; the upper mirror *B* can be rotated by the crank *D* around the vertical axis of the instrument, the angular displacement being indicated upon a divided circle *S*. The planes of the two mirrors are first placed parallel, at an angle of 45° to the vertical, and a beam of light is allowed to fall upon the mirror *A* at an angle of incidence of 57°. The reflected beam is then completely polarized and, passing upward, is reflected from mirror *B* upon the screen *C*, where it appears as a bright spot. With the mirrors parallel, the planes of incidence and reflection, and hence of polarization, coincide for each surface. Without changing its inclination, the mirror *B* with its screen *C* is rotated by the crank *D* around the vertical axis. The plane of incidence and reflection for the beams of polarized light at mirror *B* no longer coincide with that at mirror *A*; the intensity of the spot of light upon the screen accordingly begins to diminish until, after a revolution of 90°, the screen is perfectly dark, all the light being refracted and absorbed in the mirror *B*. In the latter position the planes of incidence, and hence of polarization, for

[2] The refracted rays of light are also polarized, but not completely; most of the refracted rays, however, are polarized in one direction, their plane of polarization being perpendicular to that of the reflected rays.

the light of the two mirrors are at right angles, and the mirrors are said to be crossed. By turning *D* in the same direction the spot of light reappears upon the screen, and after 180° again reaches maximum brilliancy, in which position the planes of incidence and of polarization

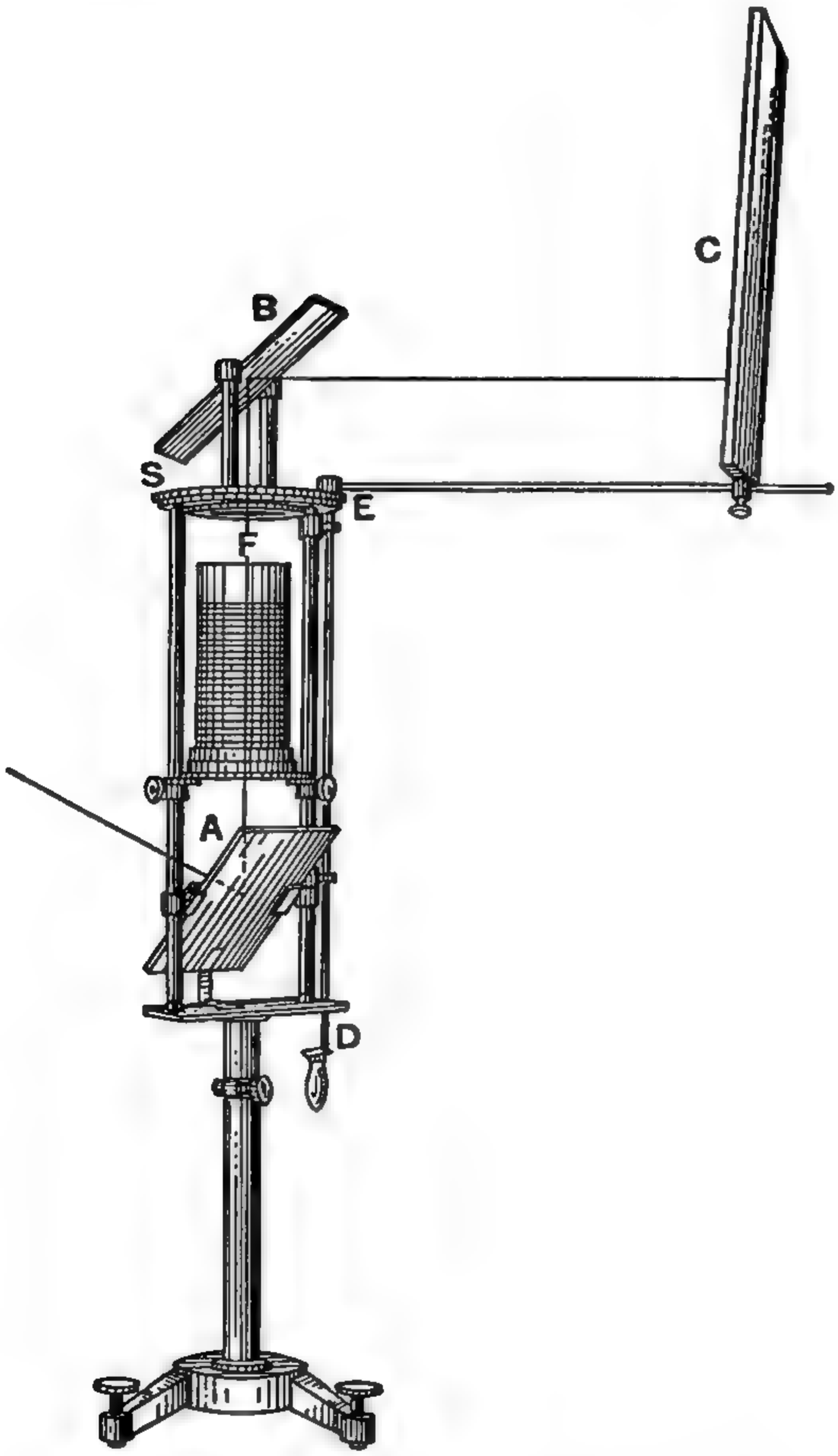


FIG. 69. Nörremberg's polarizing apparatus.

again coincide in both mirrors; at 270°, when these planes are again at right angles, the spot of light is re-extinguished.

If at one of the points of extinguishment of light upon the screen the glass cylinder *F* containing a solution of sucrose or other optical active sugar is inserted in the path of the light rays reflected from *A*,

the illumination upon the screen will reappear. The plane of polarization of the light reflected from *A* must, therefore, have been rotated by the sugar solution through a certain angle in order that reflection could take place from *B*; by turning *D* until the plane of polarization for the light upon *B* is again brought perpendicular to the plane of incidence, the point of maximum darkness is re-established. By measuring upon *S* the positions of maximum darkness, before and after the cylinder is inserted, the angle through which the sugar solution has rotated the plane of polarized light can be measured. In the Nörremberg apparatus the mirror *A* for polarizing the light is called the polarizer and the mirror *B* for measuring rotation, the analyzer.

Polarization by Double Refraction. Of the several contrivances available for producing plane-polarized light, a modified crystal of Iceland or calc spar is the only one used in the construction of polariscopes and saccharimeters.[3] Calc spar is a clear, transparent mineral

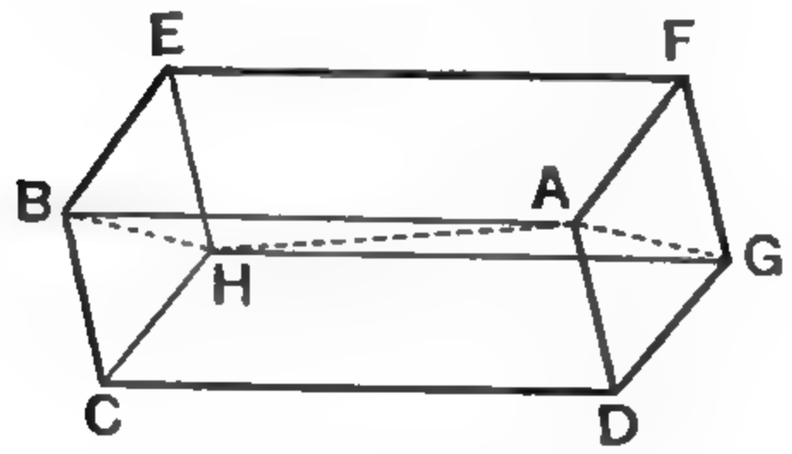


FIG. 70. Calc spar rhombohedron.

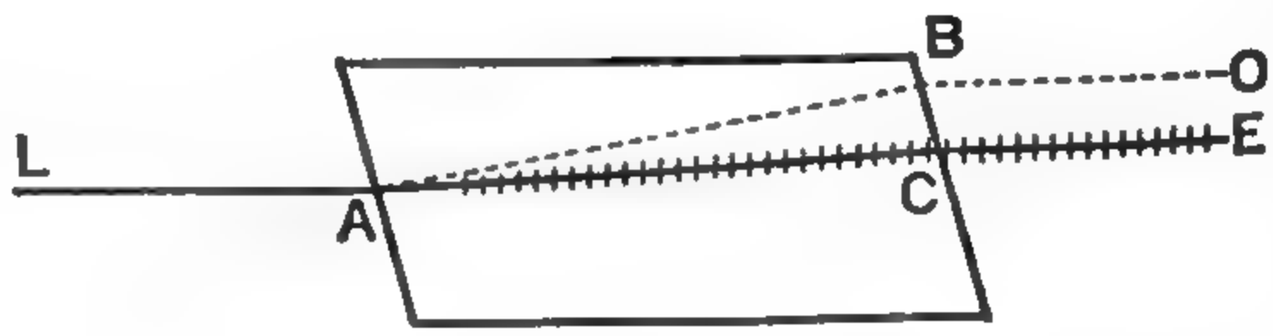


FIG. 71. Illustrating double refraction of light in calc spar.

which cleaves readily into rhombohedra. If a small object is viewed through such a rhombohedron, the image will be doubled. Rays of light in passing through the crystal undergo "double refraction." The phenomenon is noticeable in any position of the calc-spar rhombohedron except in a direction parallel to the diagonal joining the two opposite obtuse corners, known as the optical axis. Any plane including the optical axis and perpendicular to the face of the crystal is called an axial plane or principal section.

In the rhombohedron of calc spar, in Fig. 70, the direction *AH* is the optical axis. The plane *ABHG* (or any parallel plane) perpendicular to the face *AFGD* is an axial plane or principal section to that face.

If a beam of light *LA* falls upon the surface of such a rhombohedron (Fig. 71), it is resolved into two rays, the ordinary ray *ABO* and the

[3] The synthetic material "Polaroid" for which many practical uses have already been found may conceivably take the place of calcite in polarimeters and saccharimeters.

extraordinary ray *ACE*. Both these rays emerging from the crystal are polarized, their planes of polarization being perpendicular to each other.

The Nicol Prism. Before a crystal of calc spar can be utilized for polariscope construction it must be modified so as to eliminate one set of the component rays. The best-known method (that of Nicol) is the following: A rhombohedron *ABCD* (Fig. 72) is selected whose length is about three times the width. At each end of the crystal, wedge-shaped sections *BFC* and *ADE* are removed so as to reduce the acute angles *DAB* and *BCD* of the axial plane from 71° to 68°. The crystal is then divided by the plane *FGEH* perpendicular to the two modified end faces. The cut surfaces are then polished and reunited with Canada balsam.[4] The sides of the prism thus obtained are afterwards blackened and the whole is mounted by means of cork and wax in a metal tube.

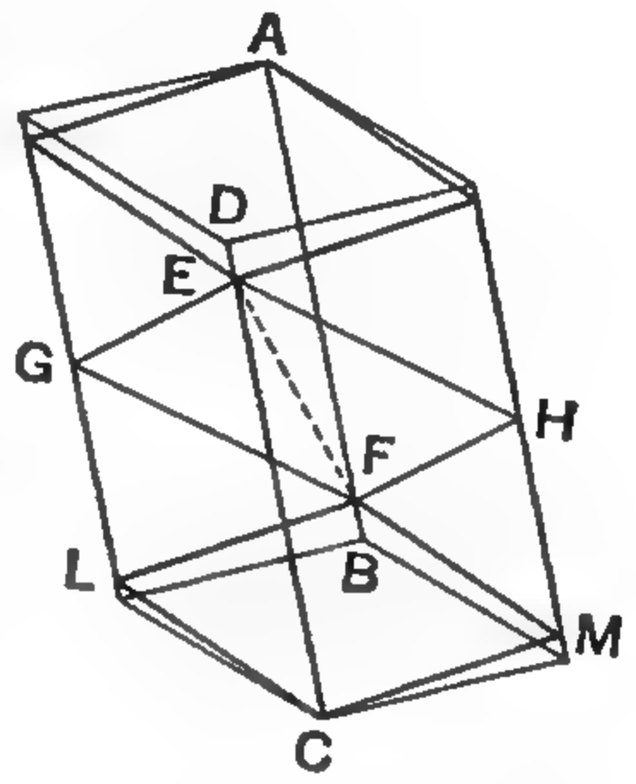


FIG. 72. Illustrating construction of Nicol prism.

Let *AFCE* represent a principal section of the Nicol prism (Fig. 73). A beam of light *LT* entering parallel to the long sides of the prism is resolved into two component rays; the component most refracted (the ordinary ray) meets the film of balsam *EF* at such an angle that it is completely reflected to the side of the prism, where it is absorbed by the dark coating. The other component (the extraordinary ray), whose vibrations are in the plane of the principal section, is less refracted and, passing through the film of balsam, emerges in a polarized condition from the end surface of the Nicol at the point *e*. With respect to the end surface of the Nicol *FCLM* (Fig. 72), the electric vibrations of the emergent light are in the plane of the principal section, i.e., in the direction of the short diagonal *FC*; the plane of polarization is in the direction of the long diagonal *LM*.

In the discussion of polarized light, it makes no difference which

[4] "Iceland spar is rather friable, and in practice it is found easier to grind away half of the rhomb instead of cutting it, as generally described. The remaining halves of two rhombs thus ground are then cemented together." — Preston, "Theory of Light," 3d ed., p. 319. According to Thompson, "Light Visible and Invisible," the crystal may be sawed by means of a copper wire and emery powder. The original Nicol prism was later modified by Nicol himself, and similar types were developed or proposed by other investigators. Linseed oil has also been used in place of balsam for uniting the cut surfaces of the prism. The original references to Nicol's work are: *Edin. New Phil. J.*, **6**, II, 83 (1829); **14**, II, 372 (1831); **27**, II, 332 (1839).

plane is taken for reference, provided it be always the same. In future pages the terms vibrate, vibration, plane of vibration, etc., refer entirely to the electric displacements in the transmission of light. With this

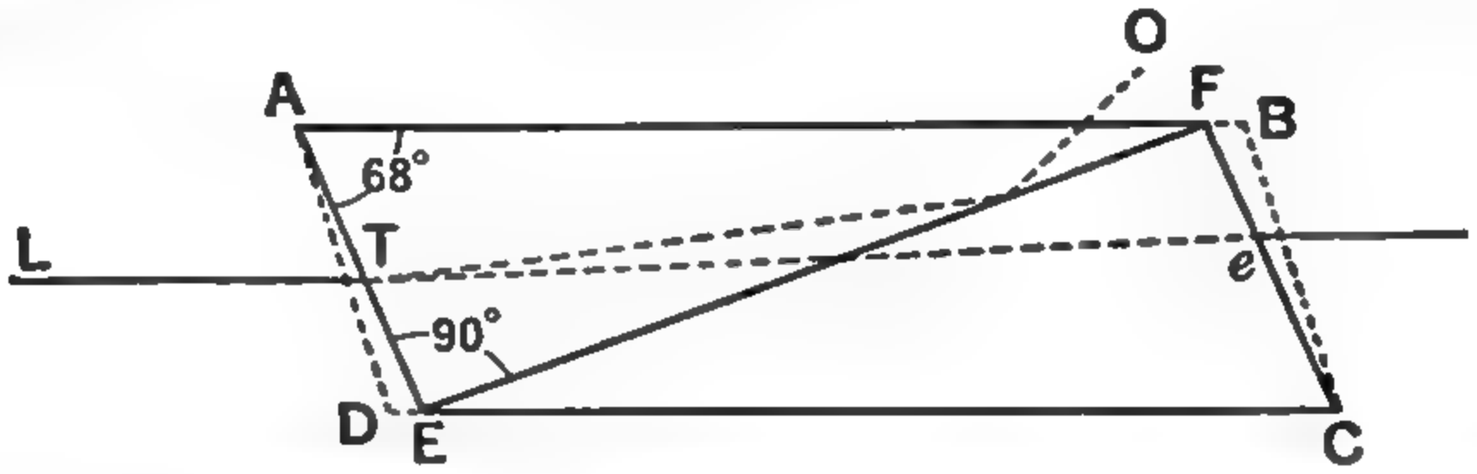


FIG. 73. Illustrating polarization of light by a Nicol prism.

understanding, the statement of Fresnel, which is followed in nearly all work upon polarimetry — that the plane of vibration of light is perpendicular to the plane of polarization — can be retained without confusion.

The Glan Prism. The type of Nicol prism which is the most scientifically perfect and the one most used at present in constructing polariscopes and saccharimeters is that of Glan. In constructing this prism the opposite obtuse corners of a calc spar rhombohedron (as *ABCDEF*, Fig. 74) are cut off by planes *PQR* and *STF* perpendicular to the optical axis which passes through the point *X*. From this section a rectangular prism *LMNO* is sawed out, which is then cut in half along a plane through *MN*. After polishing, the cut halves are cemented together again by Canada balsam and mounted as in an ordinary Nicol. The great advantages of the Glan prism over the ordinary Nicol are that the rays of light enter the prism perpendicular to the end surface and at right angles to the optical axis, thus securing the greatest amount of light capacity per unit of length.

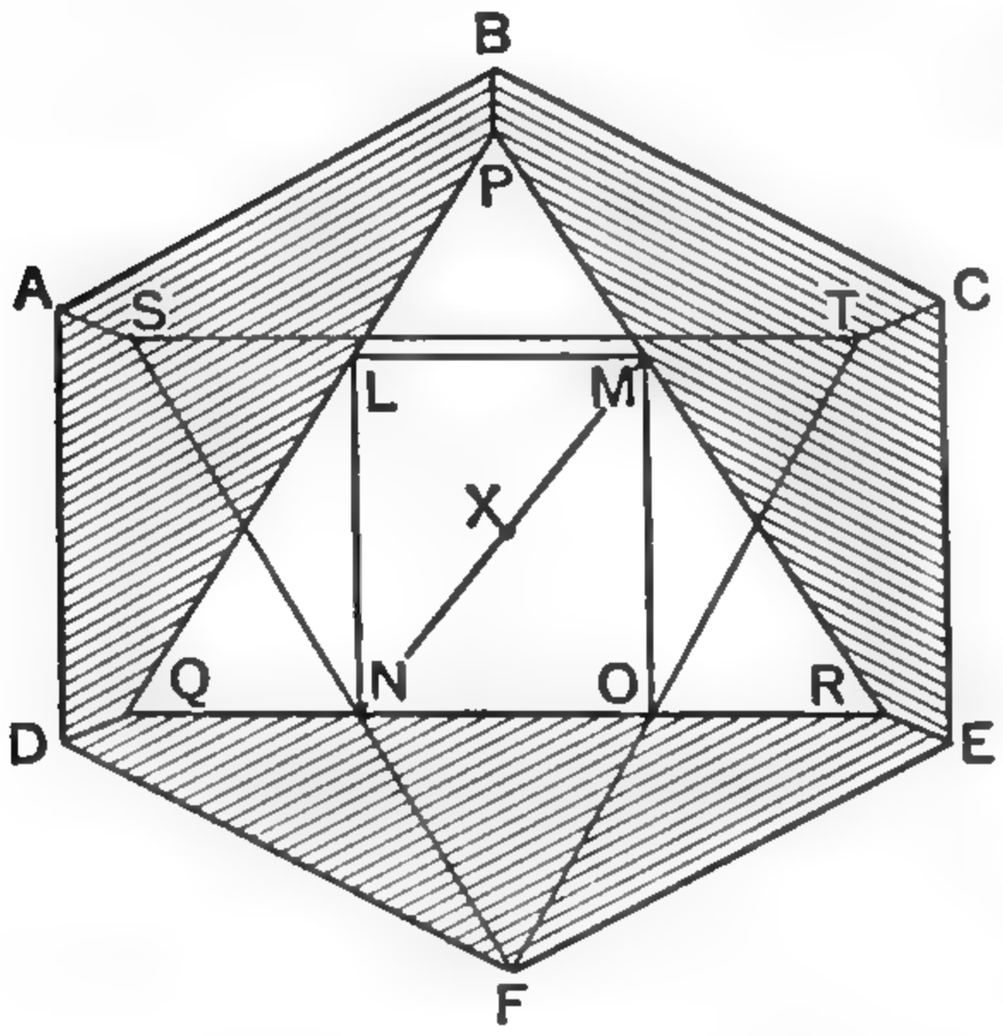


FIG. 74. Illustrating construction of a Glan prism.

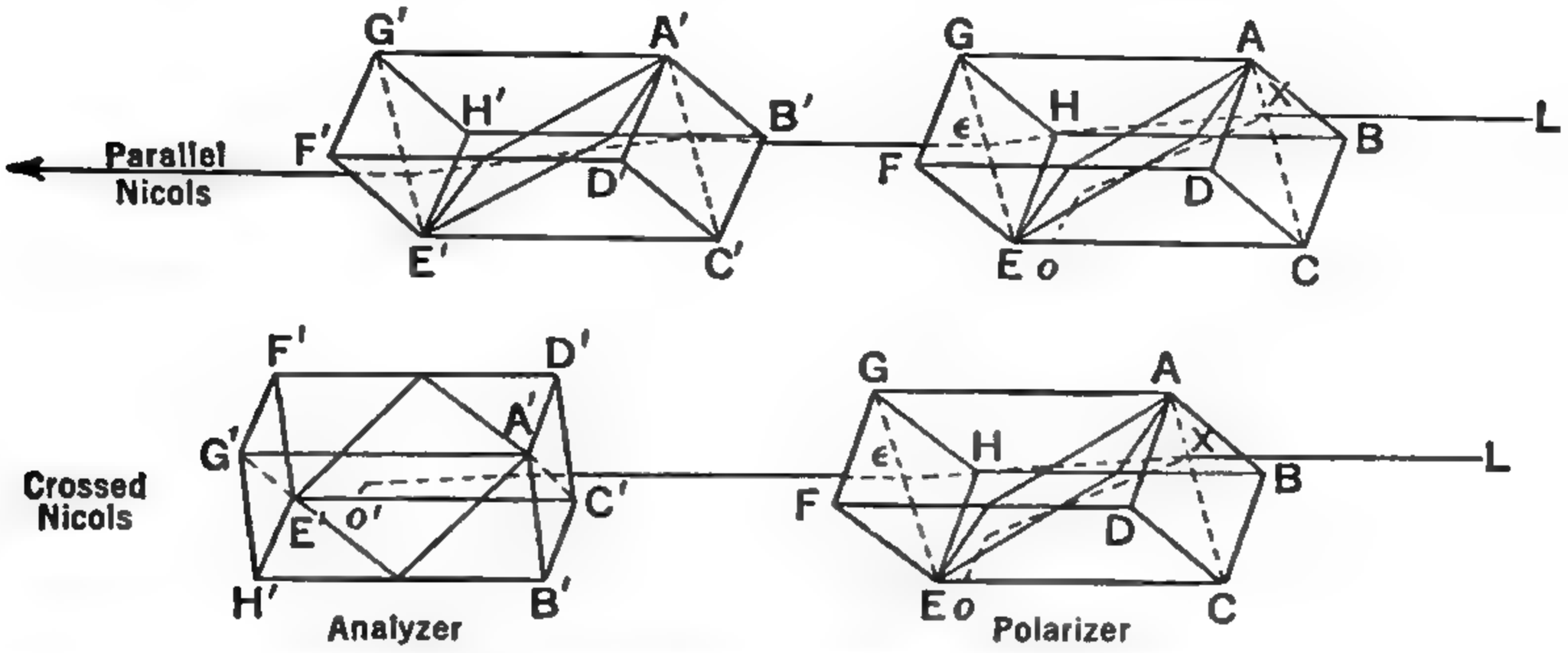
PRINCIPLE AND CONSTRUCTION OF POLARIMETERS[5]

Polarizer and Analyzer. A combination of two Nicol prisms, called the polarizer and analyzer, constitutes the essential feature of

[5] For a review of the history of the polarimeter, with references to the original literature, see Noel Deerr, *Intern. Sugar J.*, **22**, 333 (1920).

every polariscope. The function which these two parts play can best be understood from the following diagram (Figs. 75 and 76).

The polarizer, which is stationary, is represented by the prism *ABCDEFGH*, whose axial plane lies through *ACEG*. A beam of light



FIGS. 75 and 76. Illustrating principle of polarizer and analyzer. Fig. 75, *top*. Fig. 76, *bottom*.

entering from L at the point x is doubtly refracted; the ordinary rays are eliminated at o, while the extraordinary rays emerge at e, vibrating in the axial planes of the prism, with the plane of polarization parallel with the plane *BDFH*. If the emergent polarized light now enters a second prism *A′B′C′D′E′F′G′H′* (the analyzer), which can be rotated about its long axis, its course will remain unimpeded only so long as it can continue to vibrate in the same axial plane. If the analyzer is rotated about its long axis, the light which enters from the polarizer is doubly refracted and only that component which vibrates in the plane of the principal section emerges. As the analyzer is rotated the intensity of the emergent light diminishes until after a quarter revolution it is completely extinguished; in this position the axial planes of polarizer and analyzer are perpendicular to each other and the two prisms are said to be crossed (Fig. 76). If the rotation of the analyzer is continued, light will again begin to emerge, until after a half revolution, when the axial planes are again parallel, the original intensity will be restored.

The amount of light which will pass through the analyzer for any position of its axial plane with reference to the polarizer may be readily calculated by referring to Fig. 77.

Let *AB* be the axial plane of the polarizer (always stationary) and *CD* any given position of the axial plane of the analyzer, the two planes forming the angle *DOB*. From *O* lay off any distance *OP* as the

amplitude of the light emerging from the polarizer. From P erect PL perpendicular to CD; then the line OL represents the amplitude of the light extinguished in the analyzer and PL the amplitude of the light extinguished in the analyzer. As regards the relation in intensity, this is proportional to the squares of the amplitudes: $\overline{OP}^2 = \overline{OL}^2 + \overline{PL}^2$. If we erect LM perpendicular to AB and call the intensity of the light emerging from the polarizer OP, then the intensity of the light emerging from the analyzer will be represented by OM and the intensity of the light extinguished in the analyzer by MP ($OM : MP :: \overline{OL}^2 : \overline{PL}^2$). The intensities OM and OP are equal when the planes CD and AB coincide (parallel prisms); the intensity OM is 0 when the planes CD and AB are perpendicular (crossed prisms).

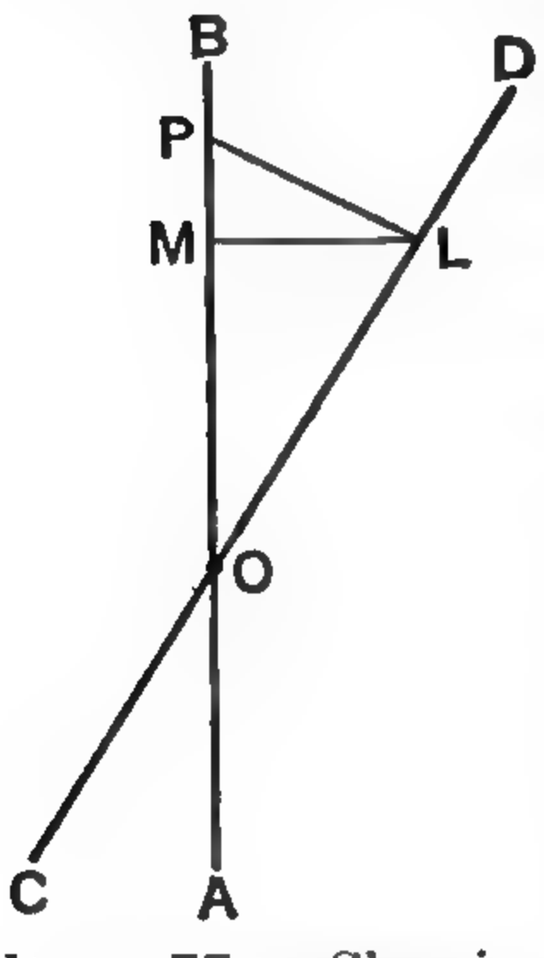


FIG. 77. Showing proportion of light extinguished by analyzer.

The construction and principle of the simplest form of polariscope can now be understood from Fig. 78. P is the polarizer consisting of a stationary Nicol, and A is the analyzer consisting of a movable Nicol mounted in a revolving sleeve; the angular rotation of A is measured upon a graduated scale S. L is the source of monochromatic light which passes through the instrument to the eye of the observer at E. We will suppose the Nicol A to be crossed with reference to P, the point of light extinction marking the 0 point on the scale S. If a tube T filled with a solution of some optically active substance, such as cane sugar, is now placed between P and A, the plane of polarized light emergent from P will be rotated

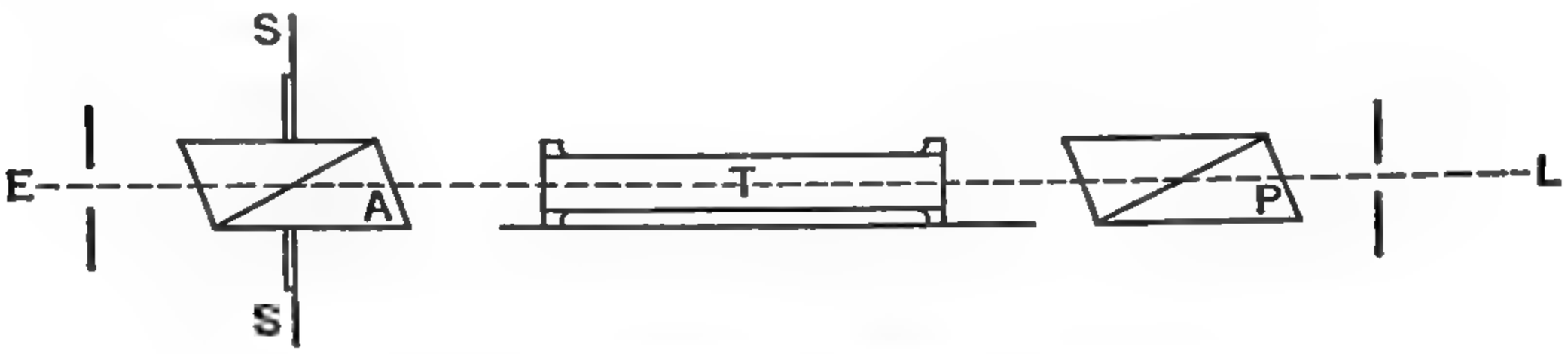


FIG. 78. Showing arrangement of parts in a simple polariscope.

from its original position and the light will no longer be entirely extinguished in A. By rotating the analyzer until its axial plane is perpendicular to the vibration plane of the light emergent from T, the point of extinction is again reached. The angular rotation of the solution in T is then determined upon the graduated scale. By continuing the revolution of the analyzer, light will again emerge from the latter,

to become re-extinguished at a point of 180° from the first reading. Owing to the fact that light rays of different wavelengths are rotated to a different extent by optically active substances (a phenomenon known as rotation dispersion), it is necessary that the light used in this type of polariscope be monochromatic.

Biot's Polariscope. The original polariscope of Biot[6] (Fig. 79), constructed in 1840, had an adjustable mirror (*M*) of black glass for the polarizer and a modified prism of calc spar for the analyzer (*A*).

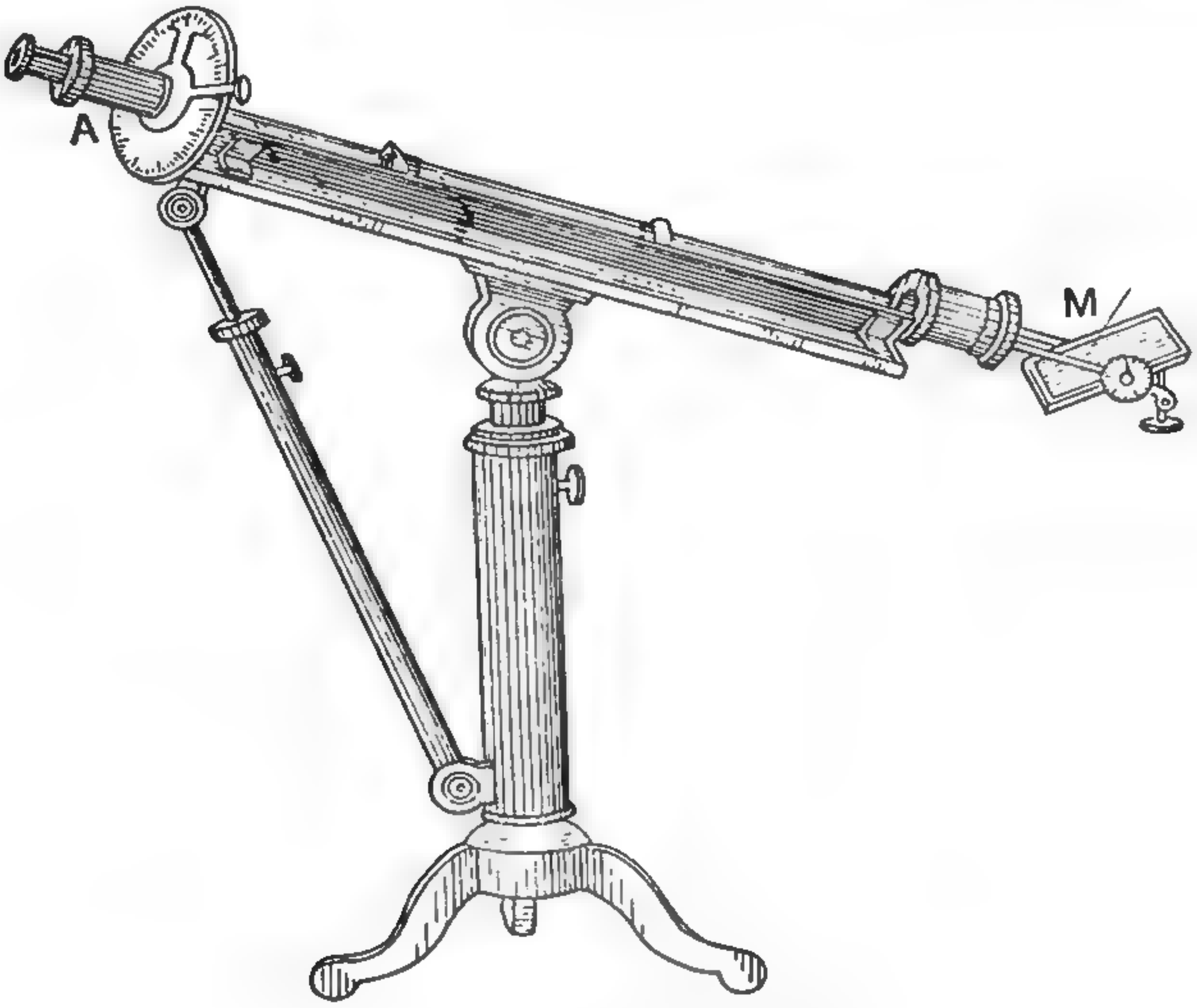


FIG. 79. Biot's polariscope.

The end point was marked by the total extinction of the extraordinary ray. The essential features of this early instrument are still retained in modern polarimeters, although in a greatly modified form.

Ventzke's Polariscope. Ventzke[7] in 1842 modified the Biot apparatus by discarding the polarizing mirror and arranging the optical parts of his instrument as shown in Fig. 78. He operated with white light, like Biot, and chose as the end point the red field appearing when the yellow rays, vibrating in a plane at a right angle with the principal plane of the analyzer, were extinguished. The circle on the analyzer was divided directly in per cent sucrose. (See p. 162.)

Mitscherlich's Polariscope. Two years later, Mitscherlich[8] introduced an instrument built on the same principles as that of Ventzke,

[6] *Ann. chim. phys.* [2], 74, 401 (1840).
[7] *J. prakt. Chem.*, 25, 65 (1842).
[8] "Lehrbuch der Chemie," 1, 361, 1844.

but he secured greater accuracy by employing monochromatic light. The field of vision was sufficiently large so that it was not completely darkened at the end point, which was marked by the appearance of a vertical black band with shaded margins. By rotating the analyzer gently to and fro until the vertical band appears exactly in the center of the field, a 0-point adjustment can be secured with a probable error of $\pm 6'$. The Biot-Mitscherlich polariscope, with position of its optical parts, is shown in Fig. 80.

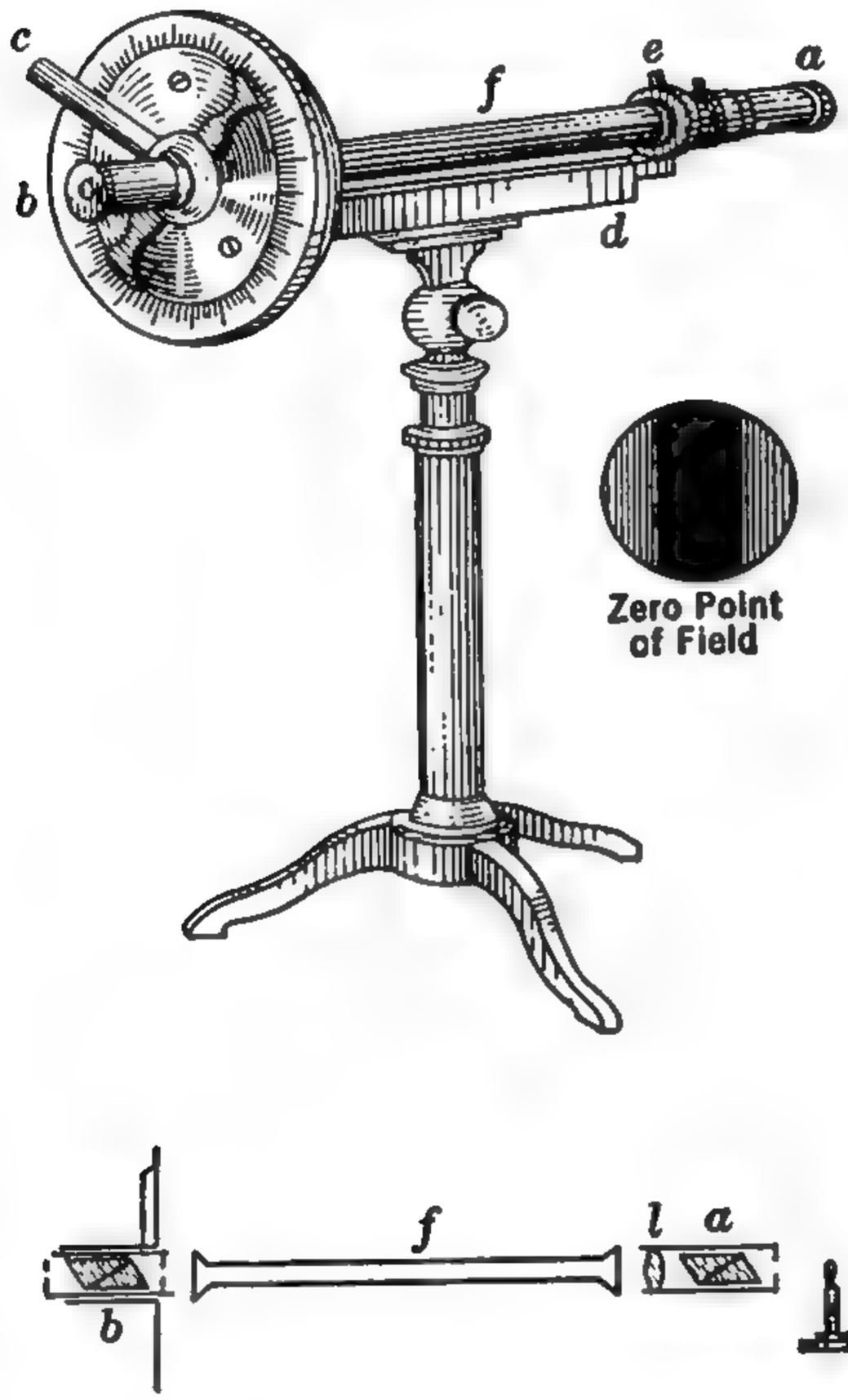


FIG. 80. The Biot-Mitscherlich polariscope.

a, position of polarizer; *b*, position of analyzer; *c*, lever for rotating analyzer; *l*, condensing lens.

Sections of the circular scales used upon the Mitscherlich and other polarimeters for measuring the angular rotation of the plane of polarized light are shown in Figs. 81 and 82. The scale in Fig. 81 for a small polariscope indicates 0.1° and is immovable, the rotation being indicated by the position of the 0 mark of the movable vernier *V*. In the illustration the 0 mark of the vernier lies between the 2° and 3° divisions of the scale; to obtain the fractions of a degree, one proceeds from the 0 mark of the vernier and, moving upward along the divisions of the main scale, comes finally to a division which exactly coincides with one of the divisions of the vernier. In the illustration this vernier division is 0.5, which, added to the reading on the main scale, makes the angular rotation 2.5°. For the larger polariscopes indicating 0.01° the main scale is movable, the circular rim divided into 0.25° rotating against the fixed vernier, which gives the readings to 0.01°. In the illustration (Fig. 82) the 0 of the vernier falls between 13.50° and 13.75°; the 0.20 mark of the vernier is in coincidence with a division on the main scale. $13.50° + 0.20° = 13.70°$, which is the angular rotation indicated.

Robiquet's Polariscope. Robiquet increased the sensibility of the Biot-Mitscherlich polariscope by introducing a Soleil double quartz

plate as the end-point device. The general appearance of this instrument, with position of optical parts, is shown in Fig. 83.

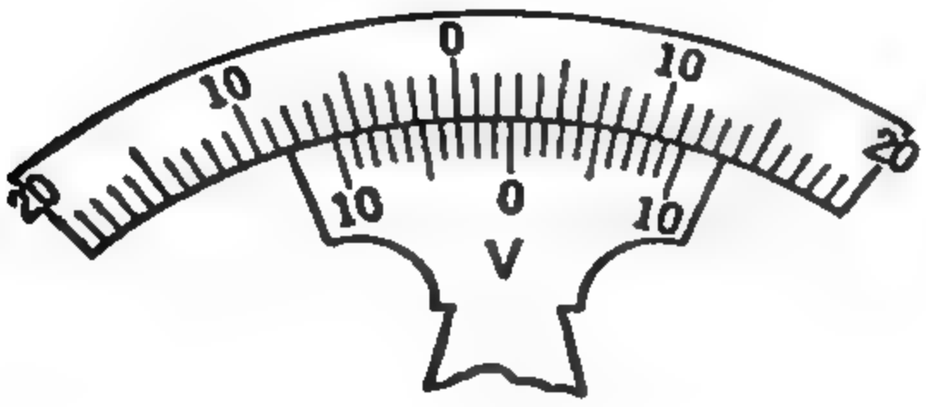


FIG. 81.

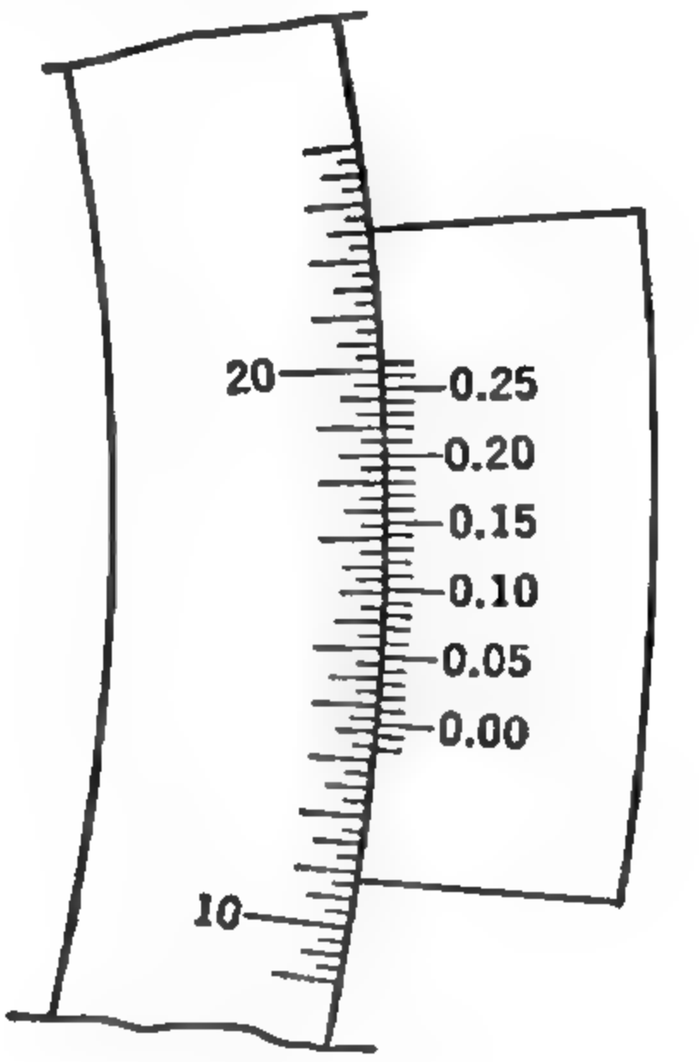


FIG. 82.

Sections of circular scales of polariscopes.

Principle of the Soleil Double Quartz Plate. The Soleil double quartz plate consists of two plates of quartz of equal thickness, one of which

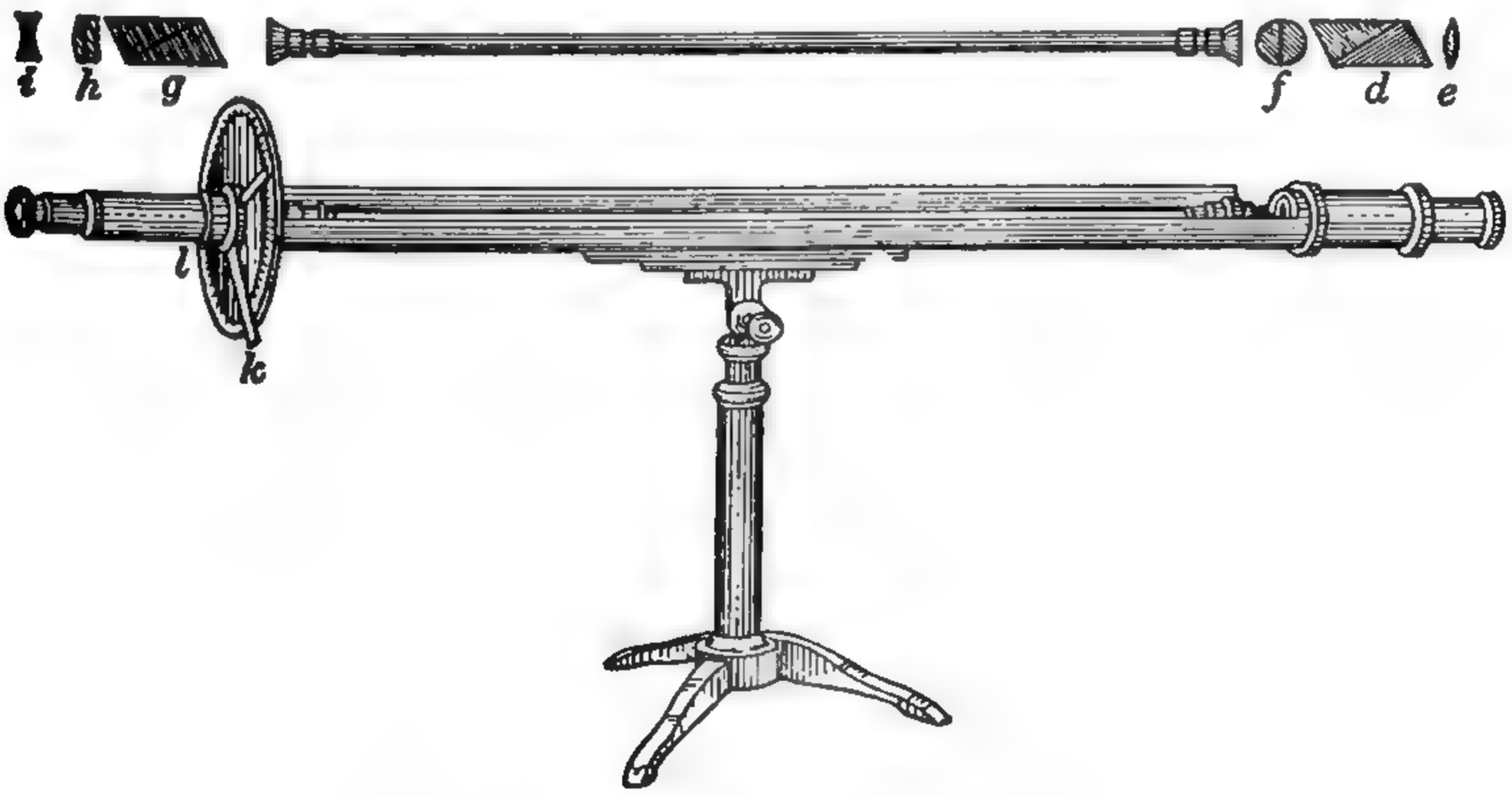


FIG. 83. Robiquet's polariscope.

d, polarizer; *e*, condensing lens; *f*, Soleil double quartz plate; *g*, analyzer; *h*–*i*, telescope; *k*, lever for rotating analyzer.

rotates the plane of polarized light to the right and the other to the left. The plates, which are cut perpendicular to the optical axis of the crystal, are cemented together at their edges and carefully ground and polished.

If white polarized light passes through such a plate, the rays of different wavelength and color will be rotated to a different degree (rotation dispersion), the rays of shortest wavelength being rotated the most. For a piece of quartz 1 mm. thick, cut as above described, the rotation will be 15.75° for the red B ray, 21.72° for the yellow D ray of sodium, and 32.76° for the blue F ray. For the average ray in the middle of the yellow spectrum the rotation is 24°. The thickness of the Soleil plate is so chosen that this average yellow ray is extinguished in the analyzer. This corresponds to a rotation of 90°, or to a thickness of 3.75 mm. (90° ÷ 24 = 3.75) for the double plate, when the end point is taken for parallel Nicols. If a plate of the above description is inserted between two parallel Nicols and examined with white light, the two halves of the field will be of a uniform rose color, the blending of the spectral colors minus the yellow. The relationship of the angular rotations for red, yellow, and blue in the two halves of a 3.75-mm. plate at the transition point may be seen from Fig. 84. By rotating the analyzer to the right or left the uniform rose color of the plate will change, one half to blue and the other to red, or vice versa. If a solution of an optically active substance is placed in the tube before the analyzer, the equilibrium in color of the transition tint will be destroyed and the two halves of the field will be differently colored. Rotating the analyzer to the point where the transition tint is again produced will give the angular rotation of the solution.

The Robiquet polariscope, which has a sensibility of about ±4′, is of course adapted only for white light. The rotation angle (α) of a substance for extinction of the mean yellow ray was expressed by Biot as α_j (j = French, *jaune;* yellow). The fact that the point j corresponds to no well-defined line of the spectrum makes it a difficult one to verify, and some confusion has resulted from this cause. Landolt gives for 1 mm. quartz, $\alpha_j = 24.5°$ instead of 24°. The value α_j is always greater than α_D (the rotation angle for the D ray of sodium). The relationship given by Landolt is $\alpha_j = \frac{24.5}{21.72}\alpha_D = 1.128\,\alpha_D$; using the value 24°, $\alpha_j = 1.105\,\alpha_D$. Many authorities employ the factor 1.111.

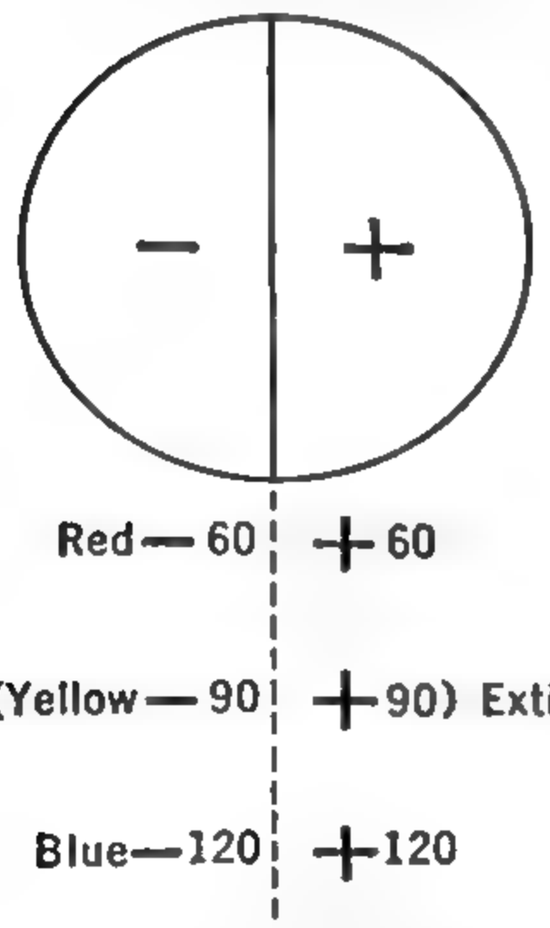


FIG. 84. Showing principle of Soleil double quartz plate.

In the examination of colored solutions, the transition tint of the Soleil double plate is affected to such a degree that a considerable error

is introduced in the observation. The use of this end-point device is valueless for the color-blind. For these reasons the transition-tint polariscopes are at present but little used.

Jellett's Half-shadow Device. Efforts to avoid the defects previously named led Jellett[9] in 1858 to devise the first half-shadow prism. This consisted of a rhomb of calc spar, about 2 inches long, squared at the ends, and divided lengthwise by a plane parallel to the edges and at a small angle with the axial plane of the prism. One of the halves was reversed with the cut surfaces in contact; the two parts were then cemented together and the ends of the prism ground and polished. The axial planes in the two halves of the prism are thus inclined, so that, when one half is crossed with the Nicol, the second half will pass light. Similarly, when the second half of the prism is brought to the point of total darkness, the first half will pass light. Midway between these positions, the two parts of the field give a uniform penumbra, or half shadow, whose depth will vary with the inclination of the axial planes in the two halves of the prism, and this is the end point (see Fig. 89). In Jellett's polarimeter, first brought out in 1864, this half-shadow prism was used as analyzer, both it and the polarizer being fixed at the end-point position. The actual measurement was made by an arrangement similar to the plunger type of colorimeter. The instrument was inclined at an angle of about 45°. Directly above the analyzer was placed a cylindrical cell, open at the top, and with a glass window in the bottom. This cell was partly filled with a liquid of opposite rotation to that of the solution to be examined. In the case of cane sugar, levorotatory turpentine was employed. The sugar solution was placed in a tube which could be lowered into the turpentine cell by means of a chain running over a spindle with milled head along a scale with vernier, until the two halves of the field of vision were balanced. The thickness of the turpentine layer was then read from the scale. The scale was first calibrated with a solution of known sugar content, and the concentration of an unknown solution could then be found from the thickness of the turpentine layer necessary to compensate for the known and the unknown. Jellett used white light, and consequently experienced difficulties from tint differences in the two halves of the field, due to differences in the rotation dispersion of cane sugar and turpentine.

Jellett-Cornu Prism. Cornu[10] modified Jellett's device and used it as a polarizer. A Nicol prism was divided lengthwise in a plane passing through the shorter diagonal at the end. A small wedge-shaped section was then removed from each cut surface and the two halves reunited

[9] *Proc. Roy. Irish Acad.*, **7**, 348 (1862); **8**, 279 (1863); *Sugar Cane*, **4**, 576 (1872).
[10] *Bull. soc. chim.* [2], **14**, 140 (1870).

(see Figs. 85 and 86). This "split" or "twin" prism combines the effect of an ordinary Nicol and Jellett prism.

The Jellett-Cornu prism was still further simplified by bisecting only one-half of the Nicol prism in the way described. The three pieces were

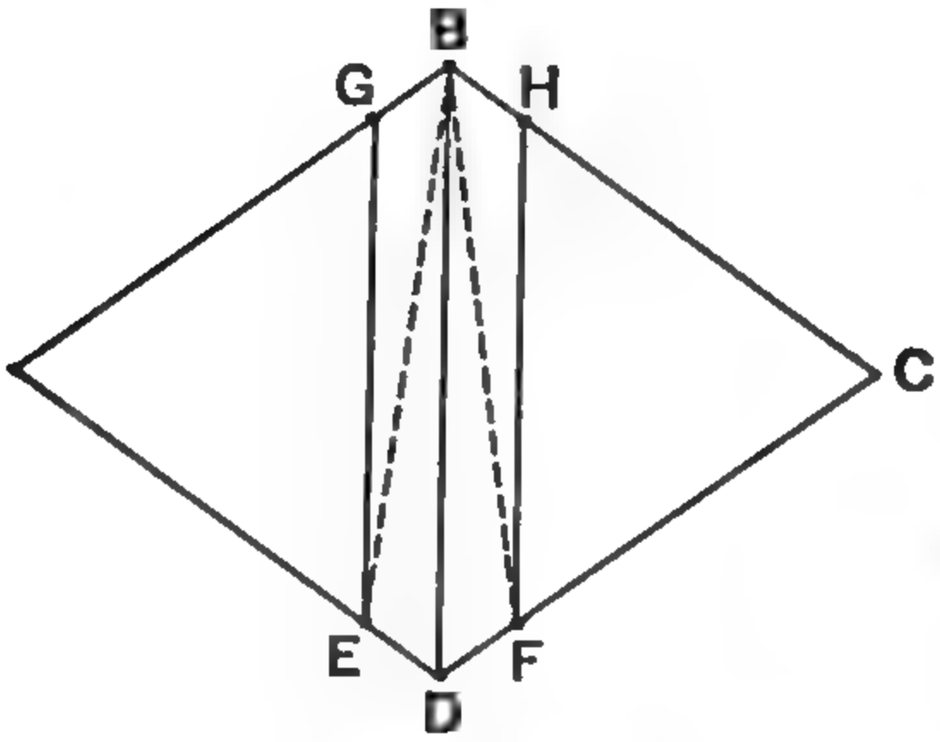


FIG. 85.

End of Nicol prism before and after splitting

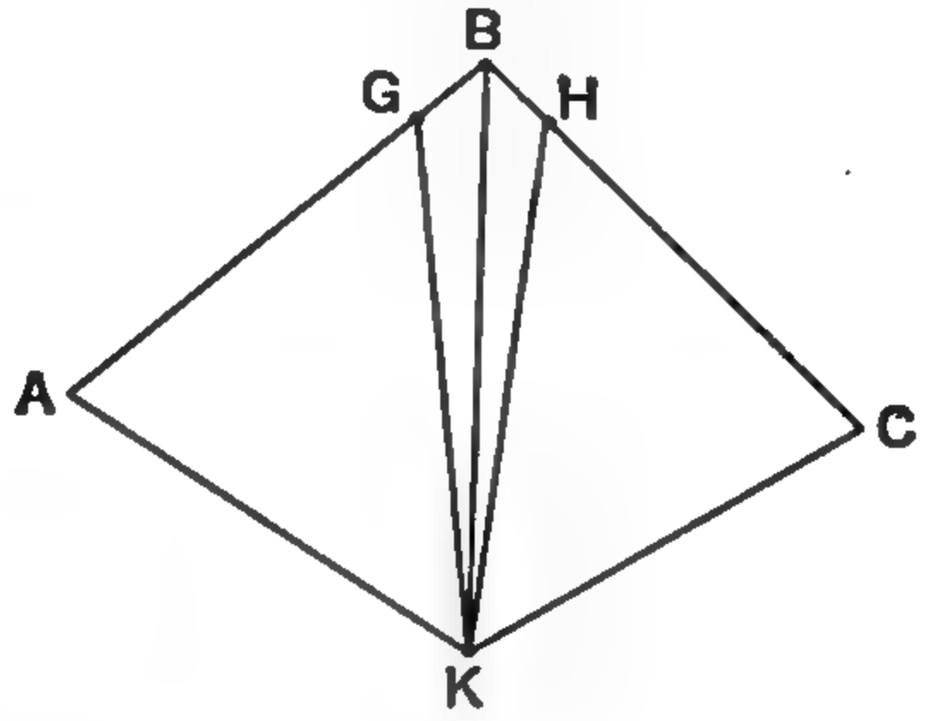


FIG. 86.

Showing construction of a Jellett-Cornu prism.

BDE and *BDF*, wedge sections removed; *GE* and *HF*, directions of axial plane before cutting; *GK* and *HK*, directions of axial planes after uniting cut surfaces.

then cemented together and the prism squared and mounted, with the split half turned toward the analyzer. This form of prism, sometimes called the Schmidt and Haensch polarizer, was formerly much used in the construction of half-shadow saccharimeters.[11]

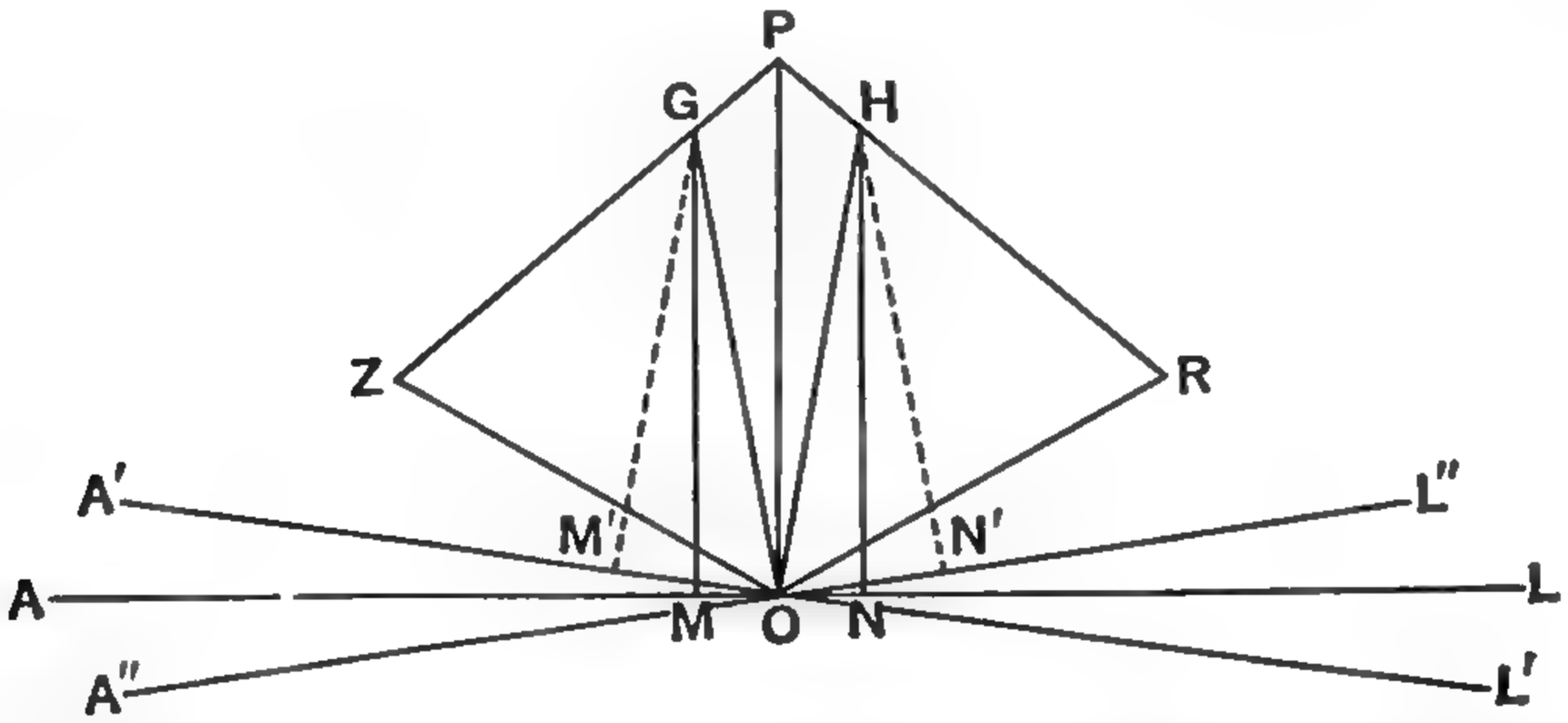


FIG. 87. Illustrating principle of Jellett's half-shadow polariscope.

The principle of the half-shadow device of Jellett and its modifications may be seen from Fig. 87.

Let *GO* and *HO* represent the directions of the axial planes in each half of the Jellett prism, forming with each other the angle *GOH* (the

[11] Landolt, "Das optische Drehungsvermögen," 2nd ed., p. 307, 1898.

half-shadow angle designated by α and made usually not to exceed 10°). It will be seen that with the axial plane of the analyzer perpendicular to *PO* the light from the polarizer will not be completely extinguished in the analyzer; a small amount of light will emerge from each half of the field proportional to the amplitudes *OM* and *ON* (see Fig. 87). The equality of light in the two divisions of the field constitutes the end point. By rotating the analyzer to the position *A'L'* perpendicular to *HO*, the light in the right half of the field will be completely extinguished, and that in the left half will be increased from *OM* to *OM'*; similarly, with *A''L''* perpendicular to *GO* the light in the left half of the field is extinguished and that in the right half increased from *ON* to *ON'*; it is evident from the above that the half-shadow angle *GOH* can be measured by the angle *A'OA''* through which the analyzer is rotated between the points of extinction in the two halves of the field. (For appearance of field at the several points see Fig. 89.)

Several types of polariscopes use the Jellett-Cornu polarizer for an end point. All have the advantage that they can be used with either mixed or homogeneous light, but the disadvantage that the half-shadow angle is fixed and cannot be changed to suit the requirements demanded by different kinds of work. The sensibility of the instrument to slight changes of rotation becomes greater as the half-shadow angle of the polarizer is made smaller; but, on the other hand, the loss of light at the end point produced by decreasing the inclination of the planes in the two halves of the field lessens the usefulness of the instrument in polarizing dark-colored solutions.

Laurent's Half-Shadow Apparatus. To overcome the last-named defect of the Jellett-Cornu polarizer, Laurent[12] in 1877 contrived an end-point device in which the half-shadow angle could be changed to suit varied requirements. The Laurent polariscope has the ordinary arrangement of Nicol prisms for polarizer and analyzer, the only difference being that the polarizer is attached to a small lever by which it can be rotated through a small angle to the right or left. The essential part of the end-point device is a thin plate of quartz cut perfectly plane and exactly parallel to its optical axis. This plate, which must be of specially prepared thickness, is mounted upon glass in such a way that it covers one-half of the field of vision. The rays of light from the polarizer on entering the plate are resolved into two components, one (the ordinary) vibrating in the plane of the optical axis, and the other (the extraordinary) in a plane perpendicular thereto. The extraordinary component, being less refracted, is transmitted more rapidly, and the thickness of the quartz plate is so regulated that, when the two

[12] *Dinglers Polytech. J.*, 223, 608 (1877).

components emerge, the extraordinary one is in advance of the ordinary by half a wavelength. The thickness of the plate depends upon the wavelength λ of the light, which must necessarily be homogeneous. The component rays which emerge from the quartz plate with half a wavelength's (or uneven multiple thereof) difference in vibration are resolved by the analyzer into light which at the end point is of the same ampli-

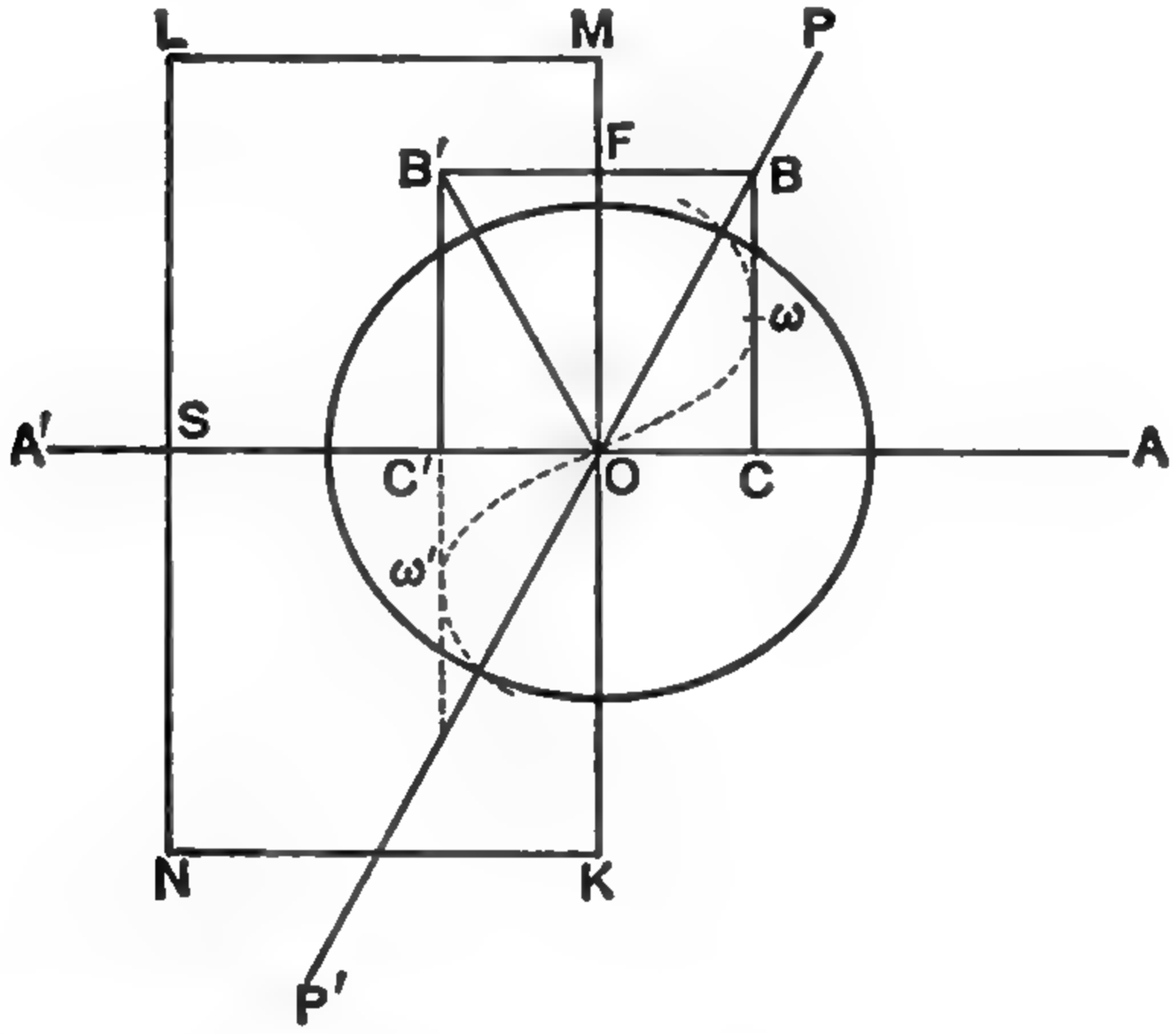


FIG. 88. Showing principle of Laurent's half-wave plate.

tude and intensity as that in the uncovered half of the field (the loss of light in the quartz plate by reflection and absorption being negligible). The two planes of vibration, which are inclined towards each other equally and symmetrically with reference to the optical axis of the plate, form the angle of the half shadow. The principle of the Laurent plate can be better understood from Fig. 88.

Let *LMNK* represent the quartz plate with the edge *MK* bisecting the circular field, *MK* being assumed for convenience to coincide with the optical axis of the plate. Let *AA′* represent the plane of the analyzer at the end point and *PP′* the plane of the polarizer, which is set at the angle *POM* with the optical axis *MK*. Lay off *OB* as the amplitude of the homogeneous light emerging from the polarizer, and draw $BC \perp AA'$; then *OC* will represent the amplitude of the light emergent from the analyzer for the uncovered half of the field. The light of amplitude *OB* upon entering the quartz plate is resolved into two components, one of which *OF* (the ordinary ray) vibrates in the plane of the optical axis *MK*, and the other *OC* (the extraordinary ray) vibrates in the plane $OS \perp MK$. The quartz plate is of such thickness

that the extraordinary component entering at the phase ω is accelerated in its passage one-half wavelength and emerges at the opposite phase ω'. The amplitude OC' being equal to OC, the resultant OB', between OC' and OF, is equal to OB, and the angle $B'OM$ equal to the angle BOM, the two together being the angle of the half shadow. The light emergent from the analyzer in both halves of the field will therefore be equal in amplitude and intensity for any angle at which PP' may be set with reference to MK. When the analyzer is rotated from its position, the equilibrium in shade between the two halves will be destroyed (Fig. 89),[13] the effect being the same as that described under Fig. 87.

I II III

FIG. 89. Showing divisions of double field of a half-shadow polariscope.

I, analyzer crossed with left half of field; II, analyzer crossed with right half of field; III, end point.

The Laurent polariscope, which is the standard instrument in France, has the great advantage, over other forms, of adjustable sensibility without change in zero point, but the great disadvantage of being adapted to monochromatic light of only one particular wavelength, because the required thickness of the half-wave plate naturally varies with the wavelength itself. In practice the Laurent instrument is always equipped with a half-wave plate for sodium light. However, by the addition of a quartz-wedge compensation system it can be converted into a saccharimeter using white light. With intense illumination and a small half-shadow angle (the conditions of greatest sensibility for all half-shadow instruments), the average error of observation according to Landolt is less than 1′.

FIG. 90.

Concentric double field.

FIG. 91.

Concentric triple field.

Concentric Half-Wave Plate. Pellin has modified the Laurent polariscope by using a half-wave plate of quartz cut in circular or annular form. The field of vision is in this way divided concentrically as shown in Figs. 90 and 91.[13] While the concentric field may secure a more

[13] In Figs. 89, 90, 91, and 95*b* the dividing lines of the fields at the end point are much intensified. With a properly adjusted instrument the dividing lines completely disappear at the end point, leaving a plain disk of uniform shade.

correct alignment of the eye with the optical axis of the polariscope, it is much more fatiguing to the eye than the ordinary bisected field. The principle of the concentric half-wave plate is the same as that of the Laurent plate.

Lippich's Half-Shadow Polarimeter. In 1880 Lippich[14] devised a form of polarizer which combines the advantages of adjustable half shadow and of adaptability to all kinds of monochromatic light. When a quartz compensation system is added, it can also be used with white light. The Lippich polarizer consists of two Nicol prisms, one large Nicol, which can be rotated about its long axis according to the needs of sensibility, and one smaller Nicol, known as the "half prism," which is mounted in front of the large Nicol so as to cover one half of the field. The half prism is slightly tilted so that its inner vertical edge forms a sharp dividing line, which can easily be focused by the eyepiece of the instrument (Fig. 92).

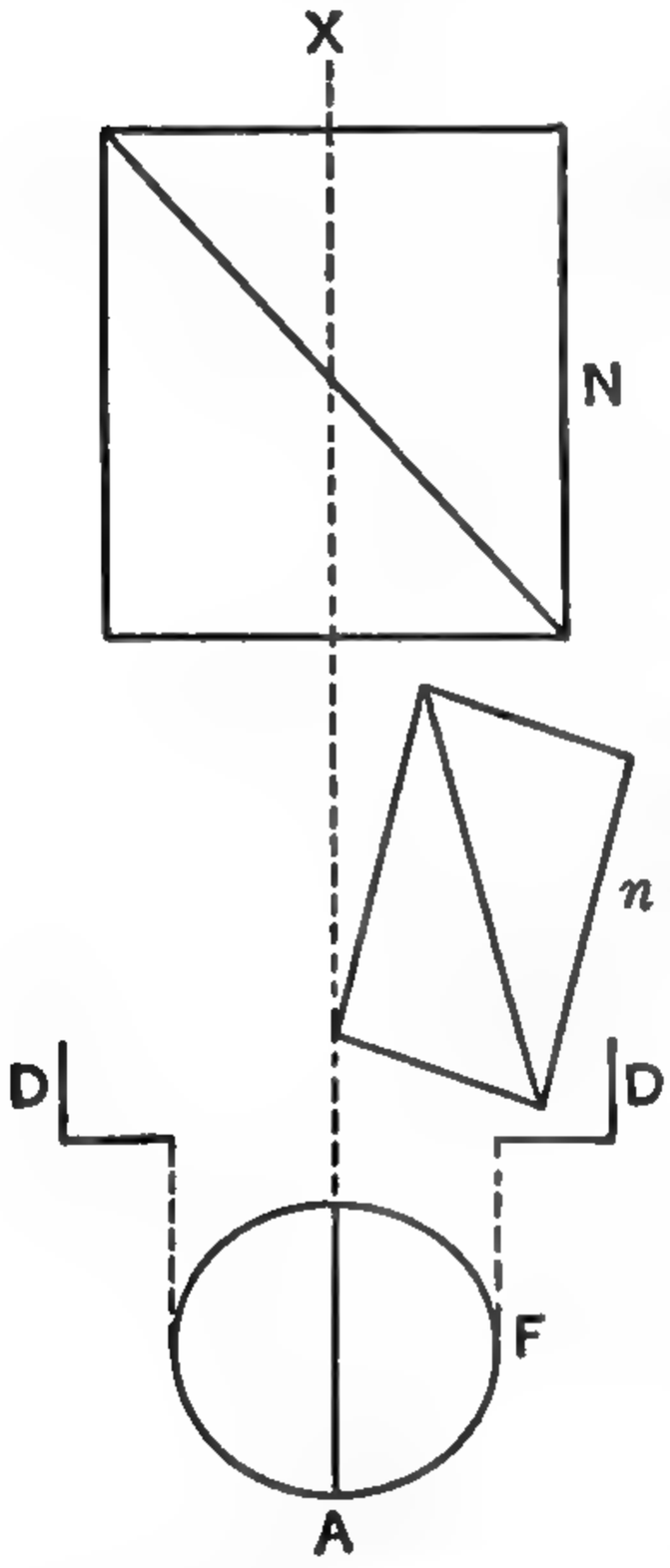


FIG. 92. Showing construction of a Lippich polarizer for double field.

N, large Nicol; *n*, small Nicol or "half prism"; *D*, margin of diaphragm; *F*, projection of field.

The principle of the Lippich polarizer can be understood by referring to Fig. 93.

Let *OP* be the plane of the large Nicol and *OH* the plane of the half prism, the included angle *POH* being that of the half shadow α. Let $OB =$ the amplitude of the light emergent from the large Nicol. Draw $BG \perp OH$. Then *OG* will represent the amplitude of the light emergent from the half prism. It can readily be seen that with a loss of a part of the light in the half prism the amplitudes *OC'* and *OD'* in the two halves of the field do not agree when the perpendicular *OA'* to the plane of the analyzer bisects the half shadow α. By rotating the analyzer slightly from *L'M'* to *LM* the amplitudes *OC* and *OD* are made equal, in which position the perpendicular *OA* no longer bisects α. The angle δ which the perpendicular *OA* makes with the bisector *OA'* will vary according to the size of the half-shadow angle α. The Lippich polarizer is therefore not symmetrical, which is a disadvantage, since by changing the half shadow α to vary the sensibility there is also a change in the 0 point of the analyzer. The latter must accordingly be readjusted for each change in sensibility.

[14] *Z. Instrumentenk.*, **2**, 167 (1882); **14**, 326 (1894).

The relation of intensities in the light emerging from the large and small prisms of the Lippich polarizer is found as follows: $\frac{OG}{OB} = \cos \angle BOG = \cos \alpha$. If I and I' are the intensities for the large and small prisms respectively, then

$$\frac{I'}{I} = \frac{\overline{OG}^2}{\overline{OB}^2} = \cos^2 \alpha \quad \text{and} \quad I' = I \cos^2 \alpha \tag{1}$$

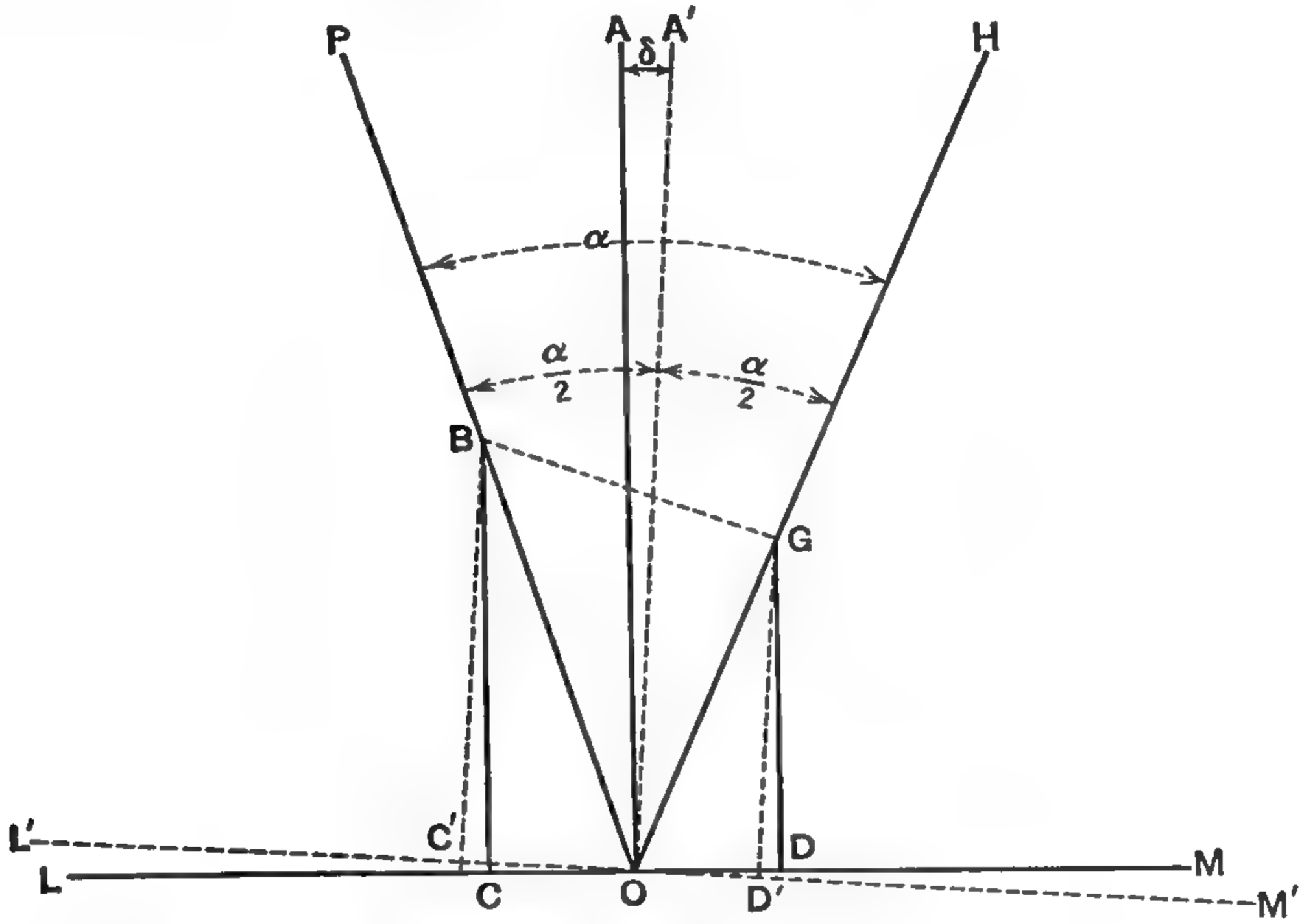


FIG. 93. Illustrating principle of Lippich polarizer.

The relation between the angle of the half shadow α and that of the change in 0 point δ may be calculated as follows: When the two halves of the field are matched the amplitudes $OC = OD$ and the intensities $\overline{OC}^2 = \overline{OD}^2$.

$$\frac{OC}{OB} = \sin \angle CBO = \sin \angle POA = \sin\left(\frac{\alpha}{2} - \delta\right)$$

$$\frac{OD}{OG} = \sin \angle OGD = \sin \angle HOA = \sin\left(\frac{\alpha}{2} + \delta\right)$$

$$\frac{\overline{OC}^2}{\overline{OB}^2} = \sin^2\left(\frac{\alpha}{2} - \delta\right) \tag{2}$$

$$\frac{\overline{OD}^2}{\overline{OG}^2} = \sin^2\left(\frac{\alpha}{2} + \delta\right) \tag{3}$$

Substituting I and I' for $\overline{OB}^2$ and $\overline{OG}^2$, we obtain

$$\overline{OC}^2 = \sin^2\left(\frac{\alpha}{2} - \delta\right) I$$

$$\overline{OD}^2 = \sin^2\left(\frac{\alpha}{2} + \delta\right) I'$$

Since $\overline{OC}^2 = \overline{OD}^2$ for the matched field, we obtain

$$\sin^2\left(\frac{\alpha}{2} - \delta\right) I = \sin^2\left(\frac{\alpha}{2} + \delta\right) I' \tag{4}$$

$$\sin^2\left(\frac{\alpha}{2} - \delta\right) = \sin^2\left(\frac{\alpha}{2} + \delta\right)\frac{I'}{I} = \sin^2\left(\frac{\alpha}{2} + \delta\right)\cos^2\alpha \tag{5}$$

$$\sin\ \frac{\alpha}{2}\cos\delta - \cos\frac{\alpha}{2}\sin\delta = \sin\frac{\alpha}{2}\cos\delta\cos\alpha + \cos\frac{\alpha}{2}\sin\delta\cos\alpha$$

Dividing by $\cos\frac{\alpha}{2}\cos\delta$, we obtain

$$\tan\frac{\alpha}{2} - \tan\delta = \tan\frac{\alpha}{2}\cos\alpha + \tan\delta\cos\alpha$$

$$\tan\delta = \tan\frac{\alpha}{2}\frac{1 - \cos\alpha}{1 + \cos\alpha} = \tan^3\frac{\alpha}{2} \tag{6}$$

In the above calculation only the light extinguished in the small Nicol has been considered. There are other factors, however, which must be taken into account in the calculation of the true 0-point correction. Schönrock[15] has shown that 7.5 per cent of the light is lost by reflection from the surface of the small Nicol, and that this amount is increased to 8 per cent or more by the loss through absorption. Equation 1 for intensity would then become

$$I' = I\cos^2\alpha\sqrt{0.92} \tag{7}$$

The value of δ thus modified would be expressed by

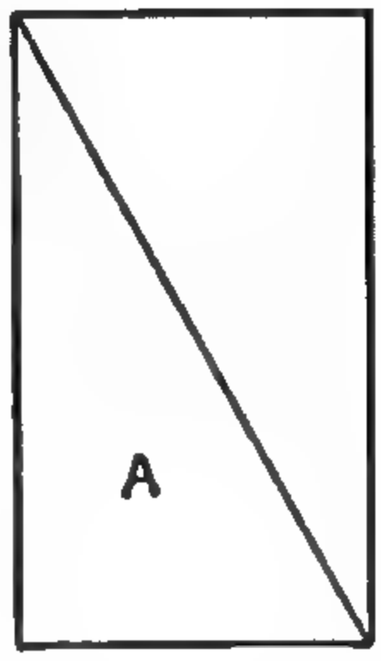
$$\tan\delta = \frac{1 - \cos\alpha\sqrt{0.92}}{1 + \cos\alpha\sqrt{0.92}}\tan\frac{\alpha}{2} \tag{8}$$

Bates[16] has shown, however, that a part of the light lost by reflection from the sides of the small Nicol is again restored in the analyzer, and that when all factors such as depolarization, size, shape, and inclination of the small prism, etc., are taken into account the true value of δ is between those calculated by equations 6 and 8, the exact figure depending upon the construction of each individual Lippich system.

Apart from the disadvantage that the 0 point must be corrected

[15] *Z. Ver. deut. Zucker-Ind.*, 58, 111 (1908).
[16] *Z. Ver. deut. Zucker-Ind.*, 58, 821 (1908).

for changes in sensibility, the Lippich polarizer is the best for general use and the one most sensitive to minute changes in rotation. The average error of adjustment, according to Landolt, with bright illumination and a half-shadow angle of 1°, is only about 15″ (0.004°).



Lippich Polarizer with Triple Field. The sensibility of the Lippich polarizer has been almost doubled by using two half prisms in place of one, the system being so arranged that the field of vision is divided into three parts (Figs. 94 and 95). The principle of the triple field can be understood by referring to Fig. 95*a*.

Let AC, ac, and $a'c'$ represent planes of the large Nicol N, and ab and $a'b'$ planes of the half prisms n and n', respectively. It will be seen that ab and $a'b'$ must be perfectly parallel in order that the half-shadow angles α and α' be equal for both half prisms, an absolute essential if perfectly uniform illumination is to be obtained at the end point. It sometimes happens that the two half prisms get out of parallelism through jarring of the instrument or expansion and contraction of the mountings. There will then be two end points for the half shadow, according to which side the middle of the field is made to agree. The observer is then obliged either to take but one of these end points, which is equivalent to reducing the instrument to an imperfect double field, or else to readjust the planes of the half prisms to parallelism, a most delicate as well as time-consuming operation. For instruments requiring constant use the increase in sensibility

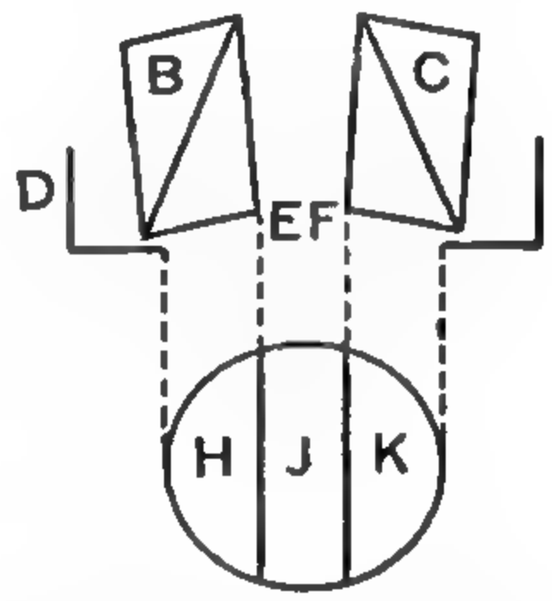


FIG. 94. Showing construction of Lippich polarizer for triple field.

A, large Nicol; *B* and *C*, small half-prisms; *D*, margin of diaphragm; *E* and *F*, inner edges of half-prism which form the divisions *H*, *J*, and *K* of the triple field.

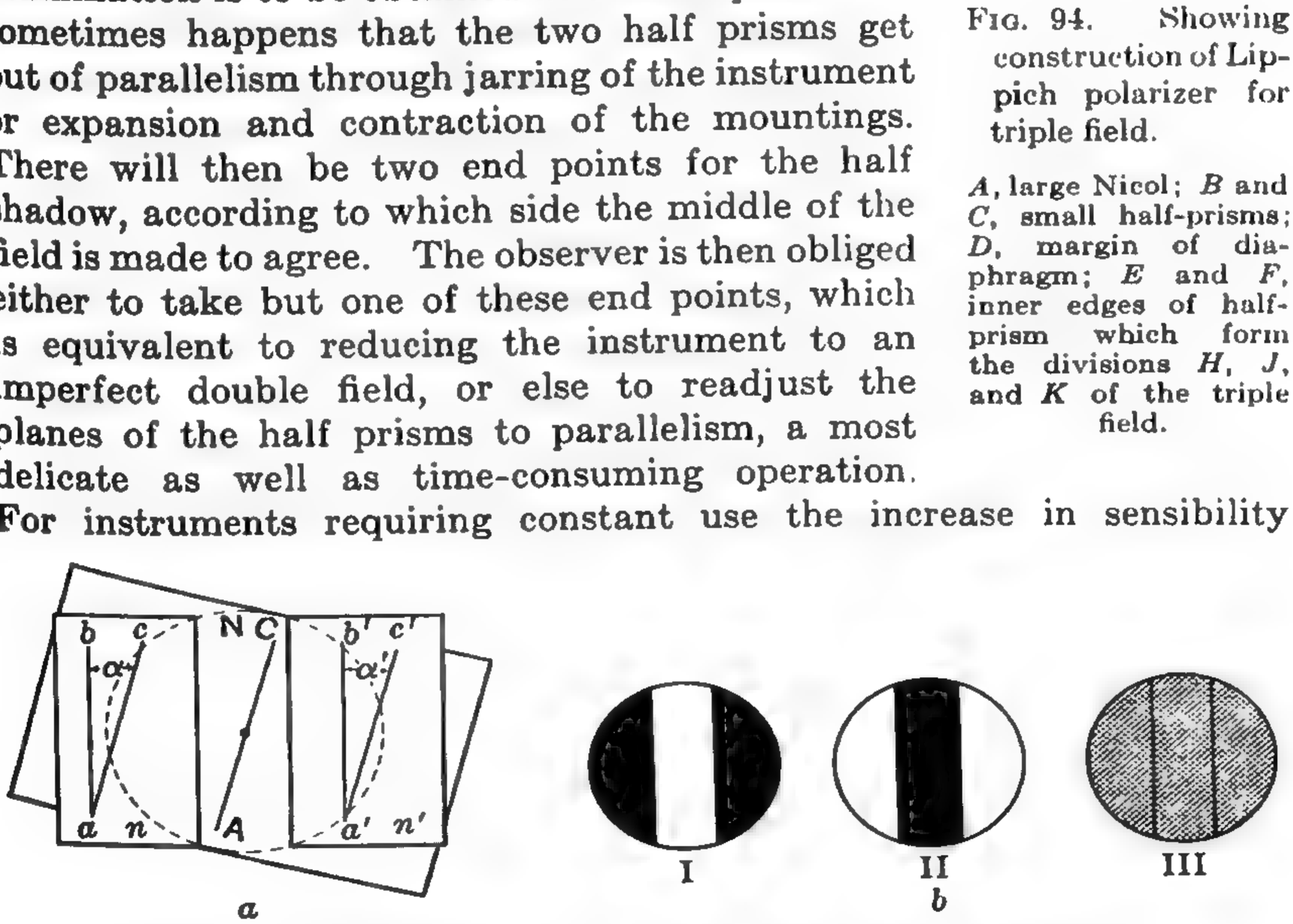


FIG. 95. Illustrating principle of Lippich polarizer for triple field.

I, analyzer crossed with outer divisions of field; II, analyzer crossed with inner division of field; III, end point.

of the triple field can hardly be said to offset the increased sensitiveness of the polarizer to disarrangement. The more simple double-field end-point device is much to be preferred for ordinary laboratory conditions.[17]

Lippich Polarizer with Quadruple Field. Lummer[18] has constructed a polarizer with quadruple field (Fig. 96) by placing before the larger Nicol *A* one large half prism *B*, and before the latter two smaller half prisms *C* and *D*. The increased complication of this form of polarizer has prevented its general introduction.

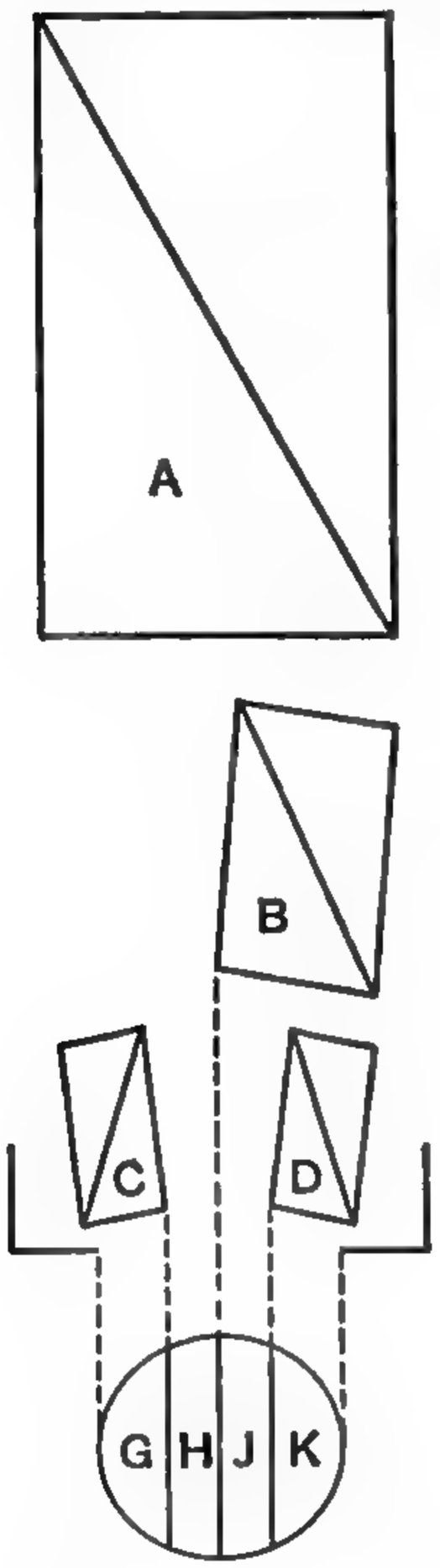


FIG. 96. Showing construction of Lippich polarizer for quadruple field.

The Lippich polarizer, besides being asymmetrical, has the disadvantage that the sharp edge of the small Nicol prism has a tendency to disintegrate, especially in the tropics, with the result that a jagged dividing line appears in the field. This difficulty has been overcome by the firm of Adam Hilger by a different manner of cutting the calcite with reference to its cleavage planes.

The Hilger Polarizer.[19] The same firm has also designed a new form of polarizer which permits a variation in the half-shadow angle without the necessity of rotating the analyzer to restore the 0 point. The light from aperture *A*, Fig. 97, is collimated by the lens *B*, and is polarized by the prisms *C* and C_1 which are slightly inclined toward each

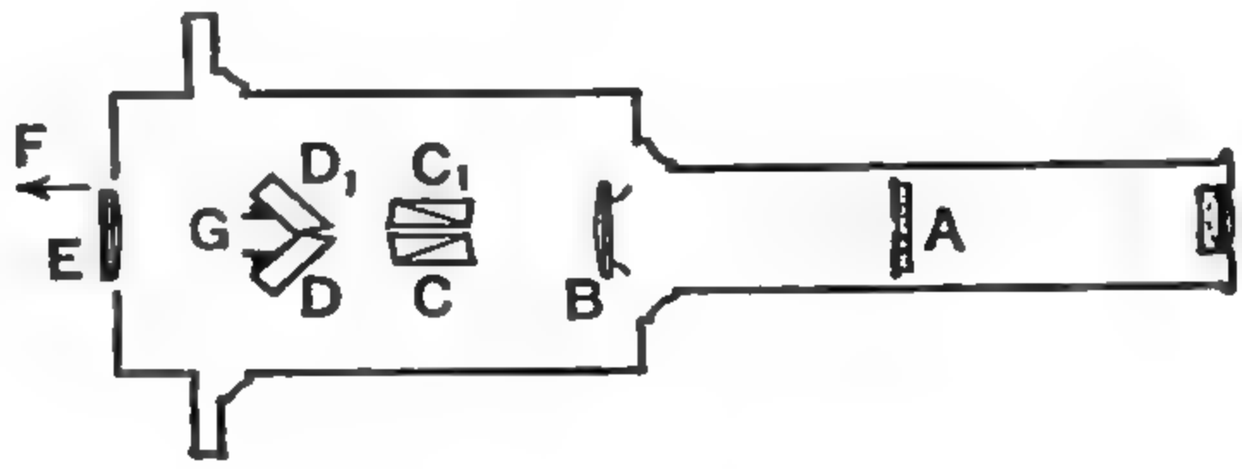


FIG. 97. Hilger polarizer.

other to form the half-shadow angle. It then passes through the parallel plates *D* and D_1 which produce a sharp dividing line at the

[17] Many chemists wrongly use the expressions half shade and triple shade in place of the terms double field and triple field. The term half shade or half shadow (German, *Halbschatten;* French, *pénombre*), refers to the depth of shade in the field at the end point and not to the division of the field. The expression triple shade is meaningless.

[18] *Z. Instrumentenk.*, 16, 209 (1896).

[19] U. K. Patent No. 166,842, 1920.

center. These plates are made of glass and are therefore quite durable. The two halves of the field are exactly alike in the amount of absorption and reflection. If a variable half-shadow angle is desired the two prisms C and C_1 are separately mounted in such a way that they can be rotated simultaneously and symmetrically in opposite directions.

Bellingham and Stanley Polarizer.[20] This device consists of two solid rhombs of calcite which are cut in such a way that the ordinary ray is absorbed at the side, while the extraordinary ray passes through. A wedge-shaped section is cut away from each rhomb to produce the half-shadow angle, and the two prisms are placed in direct contact with each other in a mounting, without the use of balsam or other cement. All such cements tend to crack after a time and to cause inequalities in the field. One of the rhombs has the sharp edge which forms the dividing line between the two halves of the field; as it is in one of the natural cleavage planes of the rhomb it is not subject to disintegration.

Other half-shadow devices have been designed by Poynting[21] and by Horsin-Déon,[22] but neither of them has come into practical use. The Ahrens polarizer[23] is used in polarizing microscopes, but not in polarimeters.

Wild's Polaristrobometer. Another form of polarizing apparatus, whose peculiarities of construction place it in a class by itself, is the polaristrobometer invented by Wild[24] in 1864. In this instrument, shown in Fig. 98, the polarizer, f, is attached to a divided circle, K, both being rotated by a rod and pinion from the screw C around the longitudinal axis of the Nicol prism. The end-point device placed at e consists of a Savart double plate made up of two sections of calc spar each 3 mm. thick, cut at an angle of 45° to the optical axis of the crystal, and cemented together so that their principal sections cross at right angles. A diaphragm c with cross threads is placed in the focus of the objective lens d of the telescope. The analyzer at a is stationary, being usually mounted with its principal section horizontal and forming an angle of 45° with the crossed sections of the Savart plate.

To determine the 0 point of the polaristrobometer, which is first illuminated at D with a sodium flame, a tube of water is placed in the instrument and the ocular of the telescope focused sharply upon the

[20] *Intern. Sugar J.*, **24**, 587 (1922).
[21] *Phil. Mag.*, **10**, 18 (1880).
[22] *Bull. assoc. chim. sucr. dist.*, **19**, 601 (1901/02).
[23] See C. A. Skinner, *J. Franklin Inst.*, **196**, 721 (1923).
[24] "Ueber ein neues Polaristrobometer," Bern, 1865.

cross threads; the field, except near the end point, consists of a series of dark horizontal parallel bands, the so-called interference fringes, which upon rotation of the polarizer increase and decrease in intensity; at certain points of rotation the bands gradually become paler until, at the maximum point of brightness, they are suddenly extinguished in the center of the field, leaving only a slightly shaded border at each

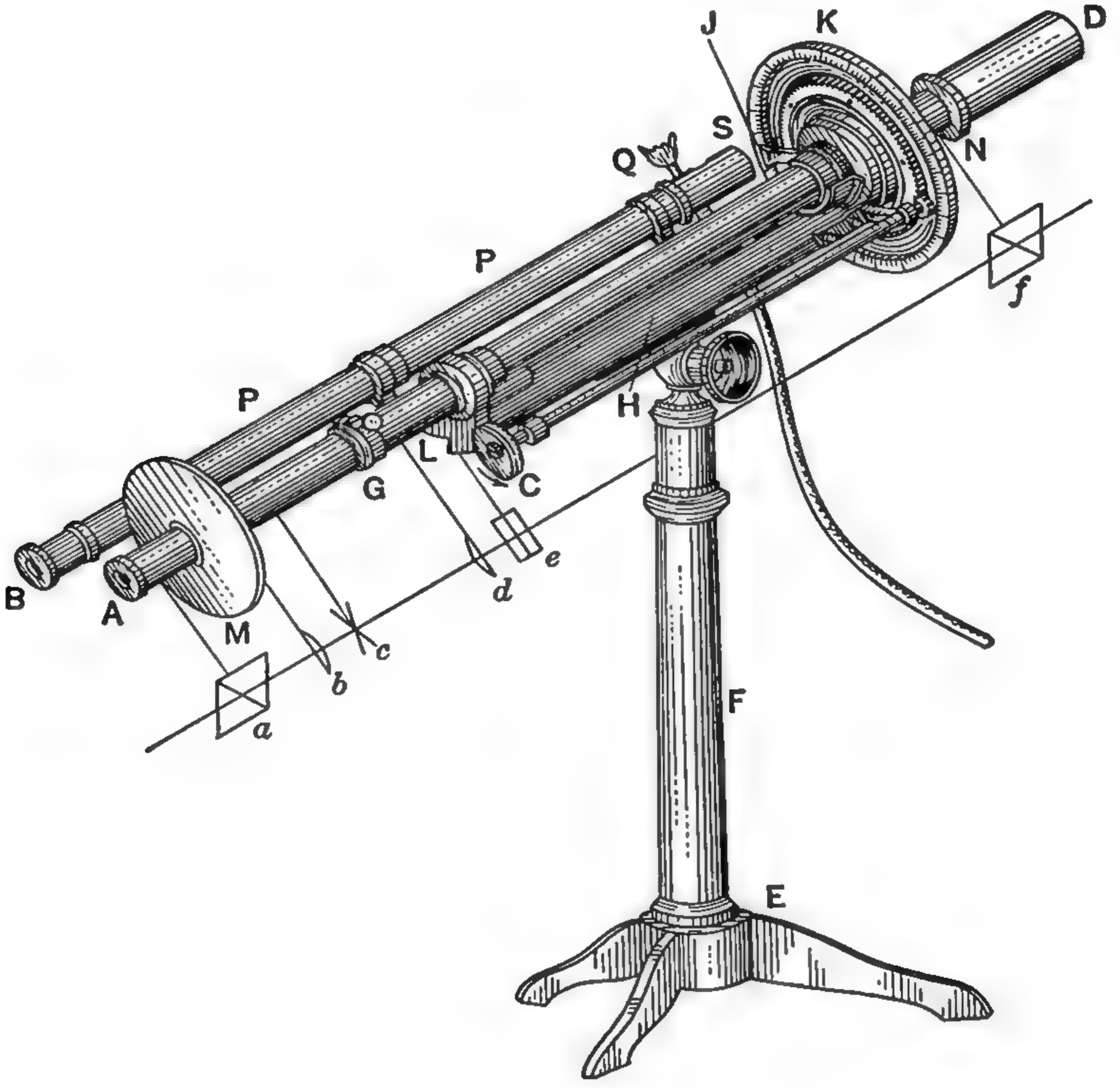


FIG. 98. Wild's polaristrobometer.

edge (see Figs. 99 and 100). The point at which the shaded borders and the extinguished part of the field are symmetrically distributed with reference to the cross threads constitutes the end point. In this position the plane of the polarizer is parallel with one of the crossed planes of the Savart plate, so that the end point reoccurs every 90°. If the extinguished part of the fringes is too wide for accurate adjustment, the intensity of the light should be diminished until the borders of the fringes are brought sufficiently close to the reticule. The

fringes have usually a different appearance at each of the end points, and also with colored solutions, so that a beginner must familiarize himself with the various characters of the field before making readings. Should the 0 points of the scale and vernier not coincide at the end point, the deviation may be noted and applied to the readings as a correction, or else they may be set at 0 and the instrument brought into adjustment by gently turning the screw *G* until the proper end point is secured.

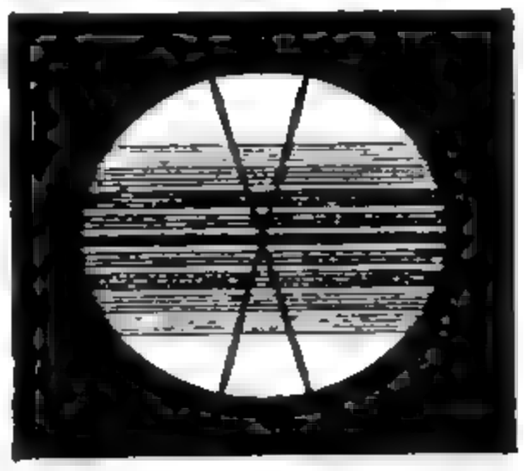


Fig. 99.

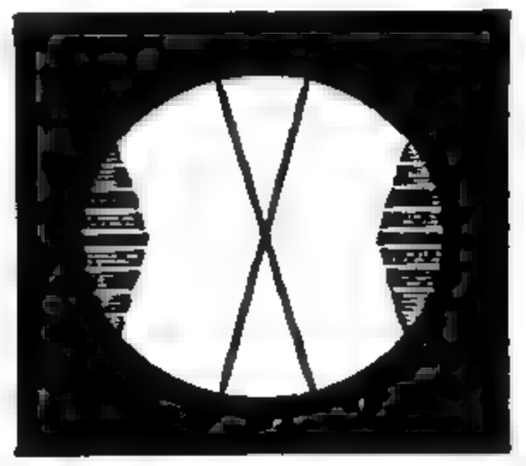


Fig. 100.

Field of Wild's polaristrobometer.

Fig. 99. Interference fringes before end point; Fig. 100. Interference fringes at end point.

If the polarizer is set at one of the four 0 points and a tube of sucrose solution is placed in the trough, the interference fringes will reappear. The polarizer must then be rotated to the left (opposite to the rotation of the sugar solution) until the fringes again disappear. The angular displacement of the polarizer to the left gives the angular rotation of the sucrose solution to the right. The readings are made through a telescope *P* which is focused upon the fixed vernier *J*; the latter is illuminated by a flame at *Q*. The average error of adjustment according to Landolt is about $\pm 3'$.

The divisions of the scale upon the Wild polaristrobometer are made usually in both circular degrees and in degrees of a sugar scale giving percentages of sucrose. The sugar scale is constructed by dividing 53.134 circular degrees into 400 equal parts. Each of these sugar divisions corresponds to the rotation of 1 g. of sucrose dissolved to 1000 ml. and polarized in a 200-mm. tube; 10 g. of pure sucrose dissolved to 100 ml. will indicate the 100° point of Wild's scale, 20 g. sucrose dissolved to 100 ml. will indicate the 200° point, 30 g. the 300° point, and 40 g. the 400° point. The normal weight of the sugar scale of the Wild polaristrobometer can therefore be varied according to the concentration of the product to be examined, the readings obtained with the 20-g., 30-g., and 40-g. normal weights being divided by 2 or 3 or 4, as the case may be. But it will be noted that the change in the specific rotation of sucrose with change in concentration is disregarded. According to Schönrock's formula for the specific rotation of sucrose, 40 g., weighed in air with brass weights, gives a rotation of 53.189°, corresponding to 400.41 parts of the Wild scale; 10 g. gives a rotation of 13.315°, equal to 100.24 parts of the Wild scale.

The Wild polaristrobometer, although formerly used in many

European laboratories, finds at present but limited application in technical sugar analysis.

De Sénarmont's End-Point Device.[25] This auxiliary apparatus which, like Savart's double plate (p. 159), utilizes interference phenomena, consists of a composite plate of quartz. It is placed between the two Nicols. When the Nicols are parallel the interference bands appear as one vertical bundle in the center of the field. But when one of the Nicols is rotated the interference bands move to one side in the upper half of the field, and in the opposite direction in the lower half of the field. This end-point device was used in polarimeters, or rather saccharimeters, constructed by Trannin,[26] and by Duboscq and Duboscq,[27] but both these instruments are now of only historical interest.

Miscellaneous Methods of Observation. The human eye is generally used to set the field of observation, within the range of the visible spectrum. If measurements are to be made in the ultra-violet region the photographic method is resorted to, and in the infra-red region the thermal effect is measured. Since the advent of radio various investigators[28] have studied the possibility of utilizing the photoelectric effect for measuring the angle of rotation, and Landt and Hirschmüller[29] have published plans for the construction of a photoelectric polarimeter.

Description of Standard Modern Polarimeters

The concluding parts of this chapter will be devoted to descriptions of a few standard forms of modern polarimeters.

Laurent's Polarimeter. As a type of instrument of French manufacture the Laurent polarimeter is shown in Fig. 101.

The 100° point of the sugar scale of the Laurent polarimeter corresponds to an angular rotation of 21.667° (21° 40′), which was originally supposed to be the rotation of a quartz plate 1 mm. thick (see discussion of French sugar scale, p. 175). The sugar scale extends 400 divisions to the right and 200 divisions to the left, thus giving ample range for polarizing all dextro- and levorotatory sugars. The normal weight of sucrose corresponding to a rotation of 21.667° had been fixed by the French government as 16.29 g., weighed in

[25] *Ann. chim.*, **28**, 279 (1850).

[26] *Assoc. Française pour l'Avancement de Science*, **1885**, 105.

[27] *J. phys.*, **5**, 274 (1886).

[28] See *Intern. Sugar J.*, **29**, 544 (1927); *Z. phys. Chem.* (B), **13**, 105 (1931); *Physik. Z.*, **37**, 1 (1936); *Compt. rend.*, **195**, 370 (1932).

[29] *Deut. Zuckerind.*, **62**, 647 (1937); also **63**, 1095, 1119, 1139, 1166, 1193 (1938).

air with brass weights, and dissolved to 100 ml. at 20° C. But in 1938 the normal weight was changed officially to 16.269 g., the figure found by Bates and Phelps. If desired, the sugar scale of the Laurent polarimeter is adjusted for a normal weight of 20 g. The circular rotation for the 100 point of this scale has been fixed at

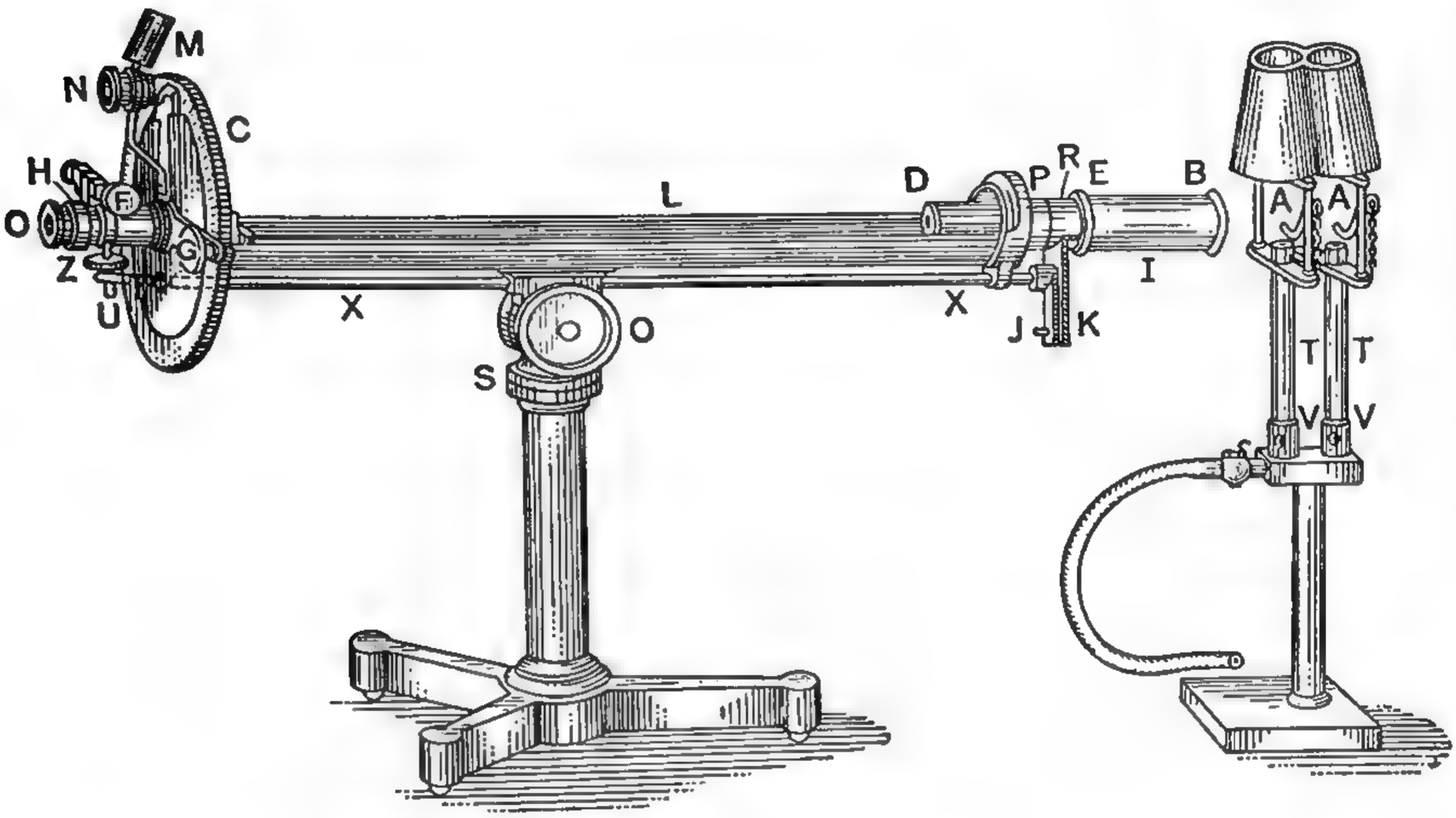


FIG. 101. Laurent's polarimeter.

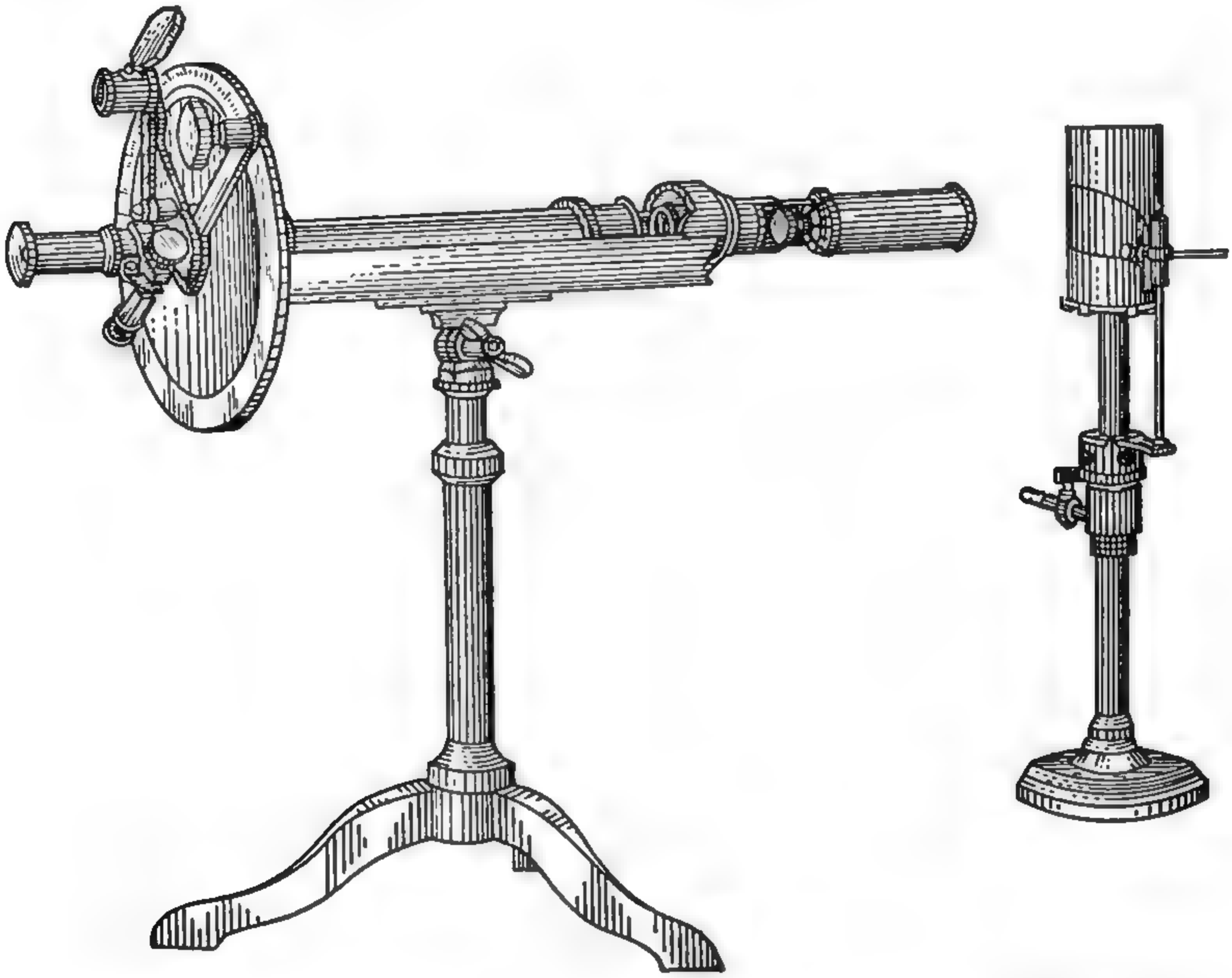
A, a duplex Laurent sodium burner placed 200 mm. from *B*; *B*, illuminating lens; *C*, quadrant whose outer circle is divided into circular degrees and whose inner circle is divided into sugar degrees; *D*, diaphragm containing half-wave plate of quartz; *E*, light filter consisting of a crystal of potassium bichromate; *F*, screw for adjustment of 0 point. *G*, geared screw for rotating the analyzer and the arm supporting the verniers; the upper vernier on the right is for reading circular degrees and the lower vernier upon the left for reading sugar degrees; *L*, bronze trough 600 mm. long for holding observation tubes; *M*, mirror for illuminating scale; *N*, magnifying glass for reading scale; *R*, tube section containing polarizer; the latter can be moved through a small angle by the arm *K*, which is moved by the crank *J* through the rod *X* by means of the lever *U*. If the solution to be examined is but little colored, the lever *U* is raised, which decreases the half-shadow angle. With colored solutions *U* is lowered until the half shadow is increased to the point of greatest sensibility. The 0 point should be redetermined after each change in the position of the polarizer.

26.60° (21.667 × 20/16.29). According to Baissac the normal weight for this standard must be reduced in the same proportion as the 16.29-g. weight, to 19.973 g., or if the 20-g. weight is to be retained, the 100° point must be fixed at 26.636 circular degrees.

Laurent instruments mounted on a trestle stand, and with a protective housing over the circle, are also made by French manufacturers.

Pellin-Duboscq Polarimeter. Another type of French polariscope is the half-shadow polarimeter-saccharimeter made by Pellin, shown in Fig. 102. The polarizer of this instrument consists of a modified Jellett-Cornu prism; the half-shadow angle is therefore fixed. The division of the quadrant into circular and sugar degrees is identical with that of the Laurent polarimeter.

The Pellin polarimeter with variable half-shadow angle (Fig. 103) makes use of a half-wave plate of quartz for the end point, which is



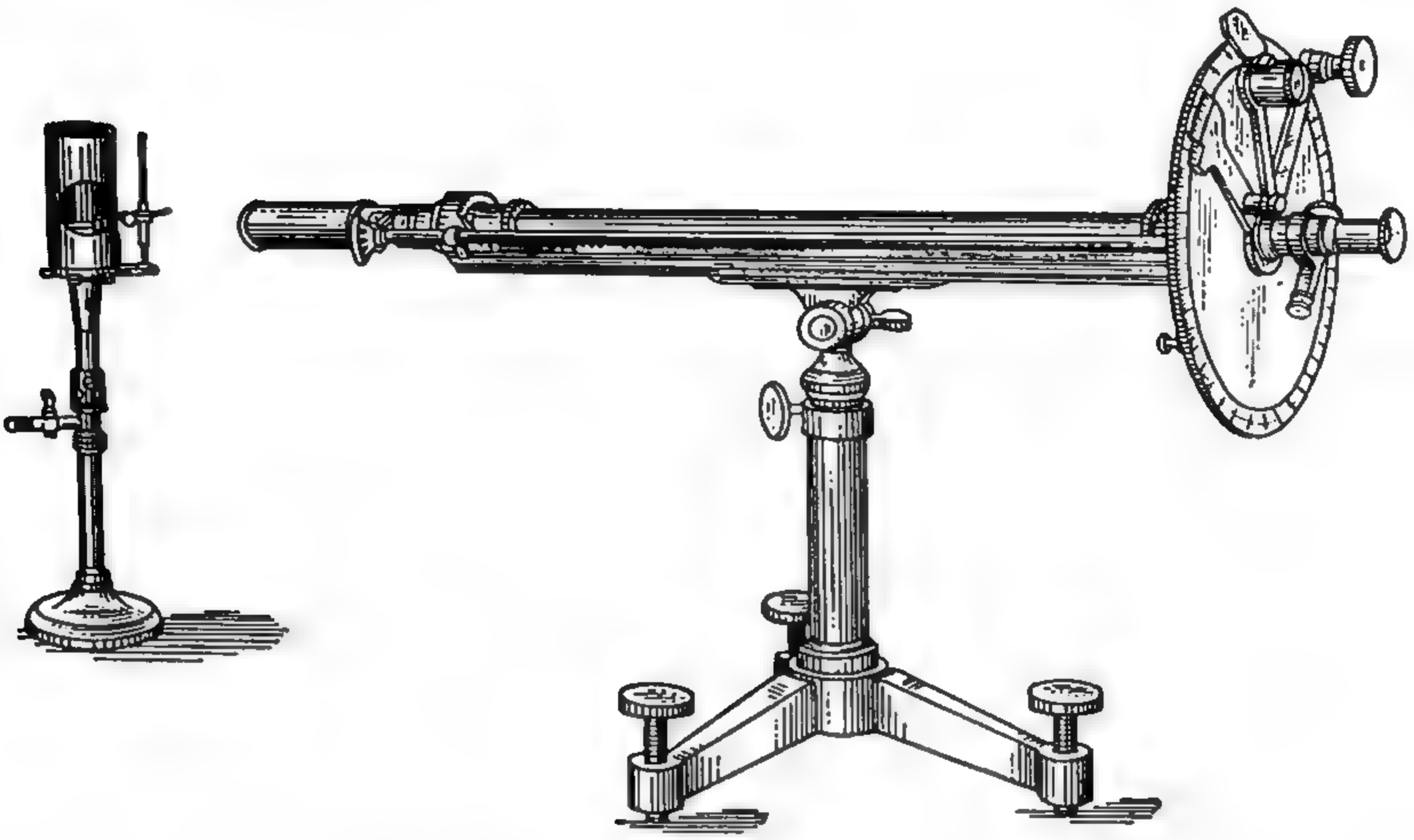
(Courtesy of Arthur H. Thomas Co.)

FIG. 102. Pellin-Duboscq polarimeter with Jellett-Cornu prism.

constructed for either divided or concentric fields. The arrangement of optical parts and method of manipulation are the same as in the Laurent polarimeter.

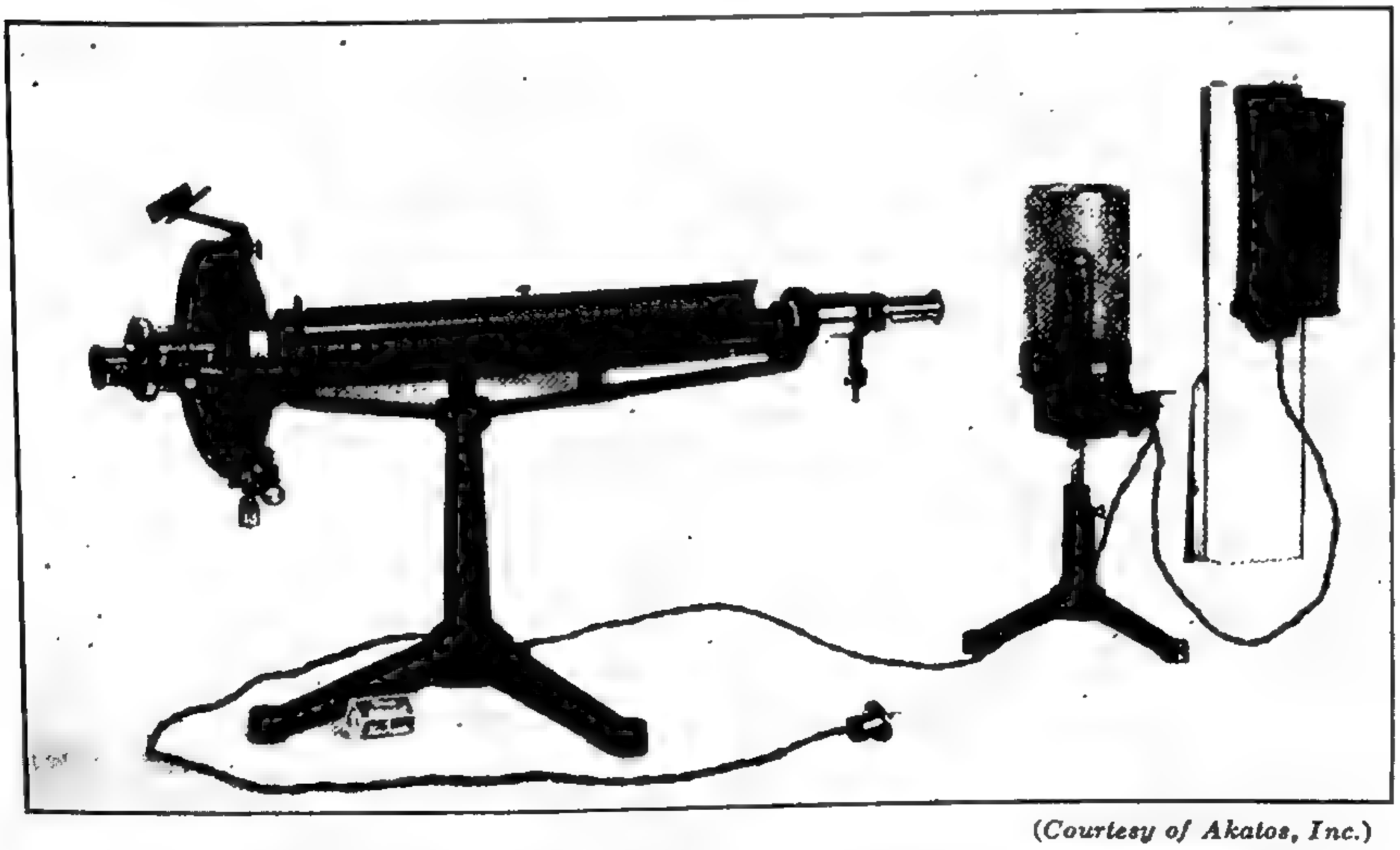
Lippich's Polarimeter. A simple form of Lippich's polarimeter adapted for general chemical use is shown in Fig. 104. Angular rotations can be measured with this instrument to about 0.01°.

A form of the Lippich apparatus devised by Landolt for more general use is shown in Fig. 105. This instrument presents an advantage in



(*Courtesy of Arthur H. Thomas Co.*)

FIG. 103. Pellin-Duboscq polarimeter with Laurent half-wave plate.

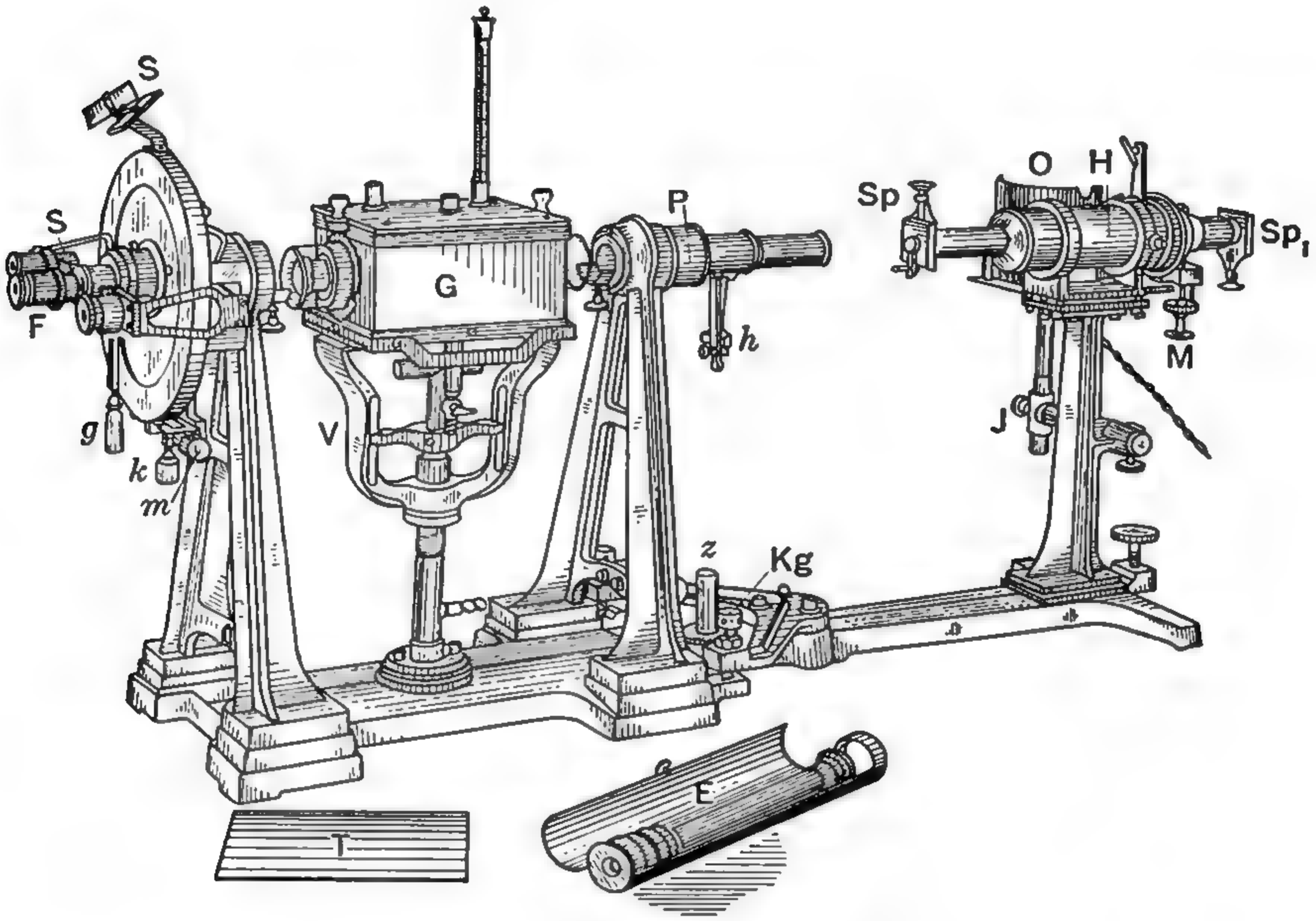


(*Courtesy of Akatos, Inc.*)

FIG. 104. Simple form of Lippich's polarimeter, with double field, illuminated by electric sodium-vapor lamp.

that any form of tube or container may be used for holding the solution or substance to be polarized. It can be read to 0.01°.

The trough *E*, for holding ordinary tubes, can be removed and the support *V* with top plate *T* employed instead. The support may be raised or lowered by means of a screw movement. For polarizing



(*Courtesy of Akatos, Inc.*)

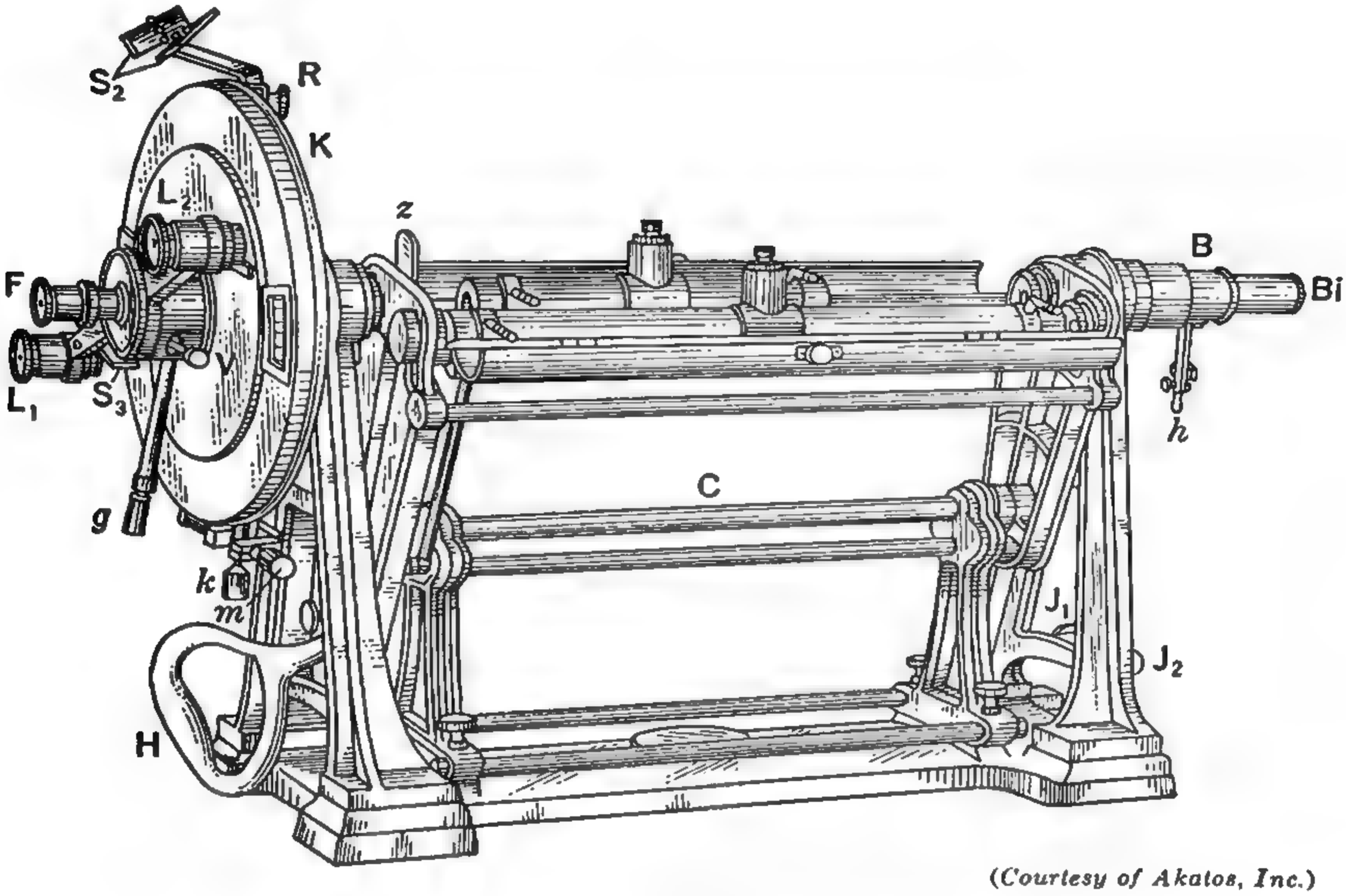
FIG. 105. Landolt's polarimeter for general use, with heating arrangement and monochromator.

g, lever for rotating the circle; the final adjustment is made by means of the micrometer screw *m* after fixing the clamp *k*; *P*, position of Lippich polarizer with two half-prisms giving triple field; *h*, lever for moving large Nicol of polarizer and regulating sensibility. The half-shadow angle which is read by the scale can be varied from 0° to 20°; *S*–*S*, mirrors for illuminating the scale, read by lenses on both sides of telescope *F*.

materials at high or low temperatures, the apparatus *G*, consisting of a polariscope tube in an asbestos-jacketed bath, is used. It is placed directly upon the support *V*, which is supplied with a gas burner. The center tube projecting through the removable top of the bath receives the overflow from the observation tube; the other tubes serve for a thermometer and stirrer for the liquid in the bath. For polarizing at low temperature a cooling medium is used in the bath, in which case the ends of the observation tube must be covered

with desiccating caps to prevent condensation of moisture upon the cover glasses. Electrically heated baths of various designs are also obtainable. The stand *V* and top *T* may also be used as a support for troughs or holders of any desired shape, to read either solutions or solids.

The special feature of another polarimeter, devised by Landolt and shown in Fig. 106, is the double trough by which different tubes



(*Courtesy of Akatos, Inc.*)

FIG. 106. Large model Landolt polarimeter.

filled with solution can be brought into the field by movement of the large lever *H*, for rapid comparisons.

The universal polarimeter of Lippich, Fig. 107, is used for work of the highest precision. It takes tubes up to 1 meter in length, and the circle can be read to 0.001°. This requires very close temperature control, effected by the large asbestos-covered jacket *E*. The polarizer is equipped with two half prisms which can be adjusted separately in every direction by levers and worm gears. The position of the polarizer prisms can be read on special scales. The light must be carefully purified in order to utilize the high precision made possible by the graduations on the analyzer circle. For this reason the instrument is permanently fitted with a monochromator.

The Gaertner Polarimeter. This instrument, Fig. 107*a*, manufactured in the United States, is similar to the Lippich polarimeter described on p. 164. It is mounted on a heavy trestle stand and accommodates tubes up to 400 mm. in length. The trough can be removed and replaced by other supports for special purposes. The polarizer is of the triple-field Lippich type, and the analyzer consists of a Glan-Thompson prism. The circle is completely protected by a cover with two glass windows for viewing the scale which may be read to 0.01°, by means of small lamps provided for the purpose.

Polarimeters similar to those described are furnished also by J. and J. Frič in Czechoslovakia, by Bellingham and Stanley and by Hilger in England, and by others. The principal difference between instruments supplied by the various manufacturers is usually in the type of polarizer employed (see pp. 154–159); otherwise polarimeters of the same precision differ generally only in constructional details.

Polarimeters to be used for research on electromagnetic rotation are constructed entirely of materials free from iron.

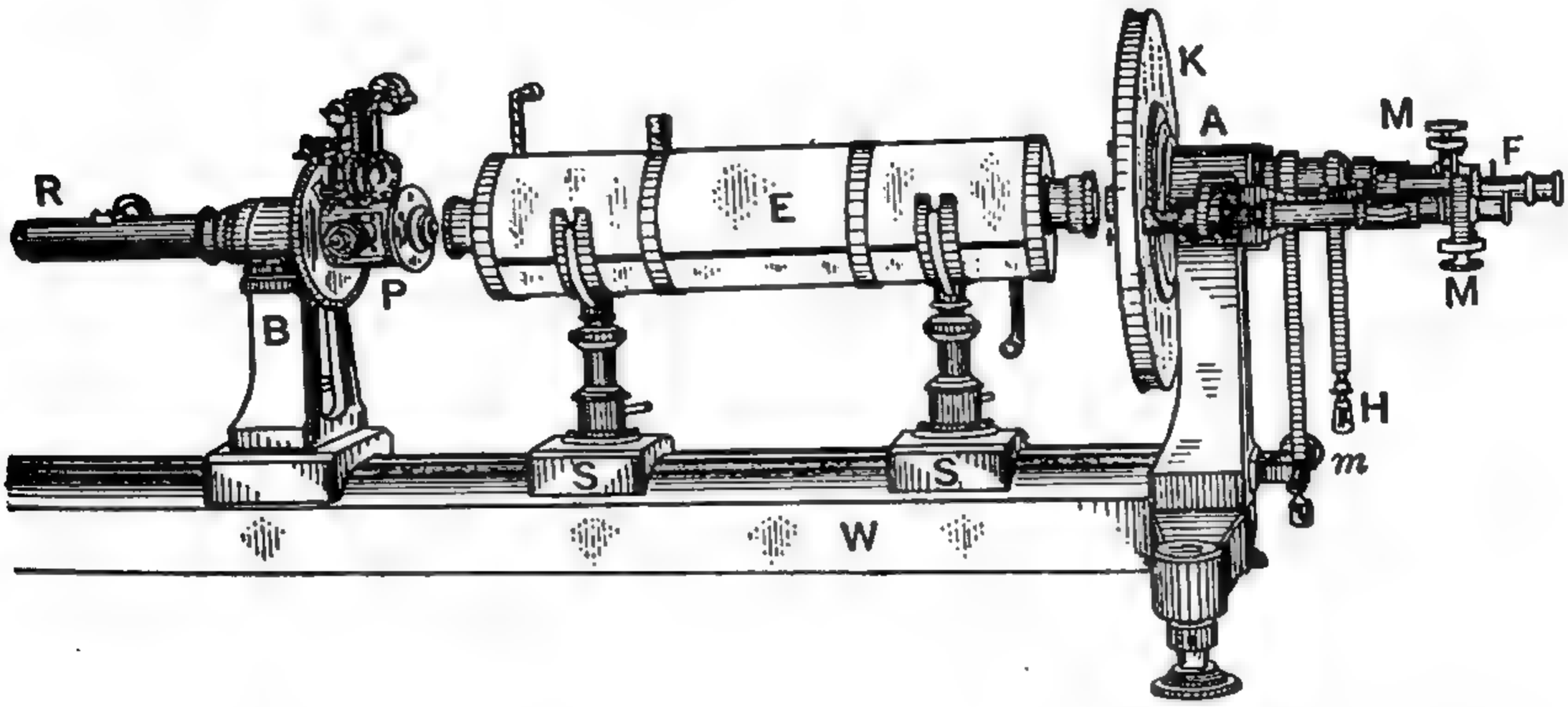
Besides the Laurent polarimeters described on pp. 162–163, instruments with an auxiliary cane sugar scale engraved on the circle have been obtainable for many years. But they were not well adapted for routine sugar analysis because of the low intensity of the usual gas sodium burner and the difficulty in keeping the burner supplied with sodium salt. The modern electric sodium-vapor lamp (p. 233) has made it possible, however, to dispense with the expensive quartz-wedge saccharimeter and to use a polarimeter instead. A few such polarimeters used as saccharimeters are described in Chapter VI, pp. 224–227.

Polarimeters of simplified construction, with restricted range and lower in price, are manufactured for special purposes, such as the determination of glucose and albumin in urine by measurement of the rotation of the urine before and after boiling.

Verification of Scale Reading of Polarimeters

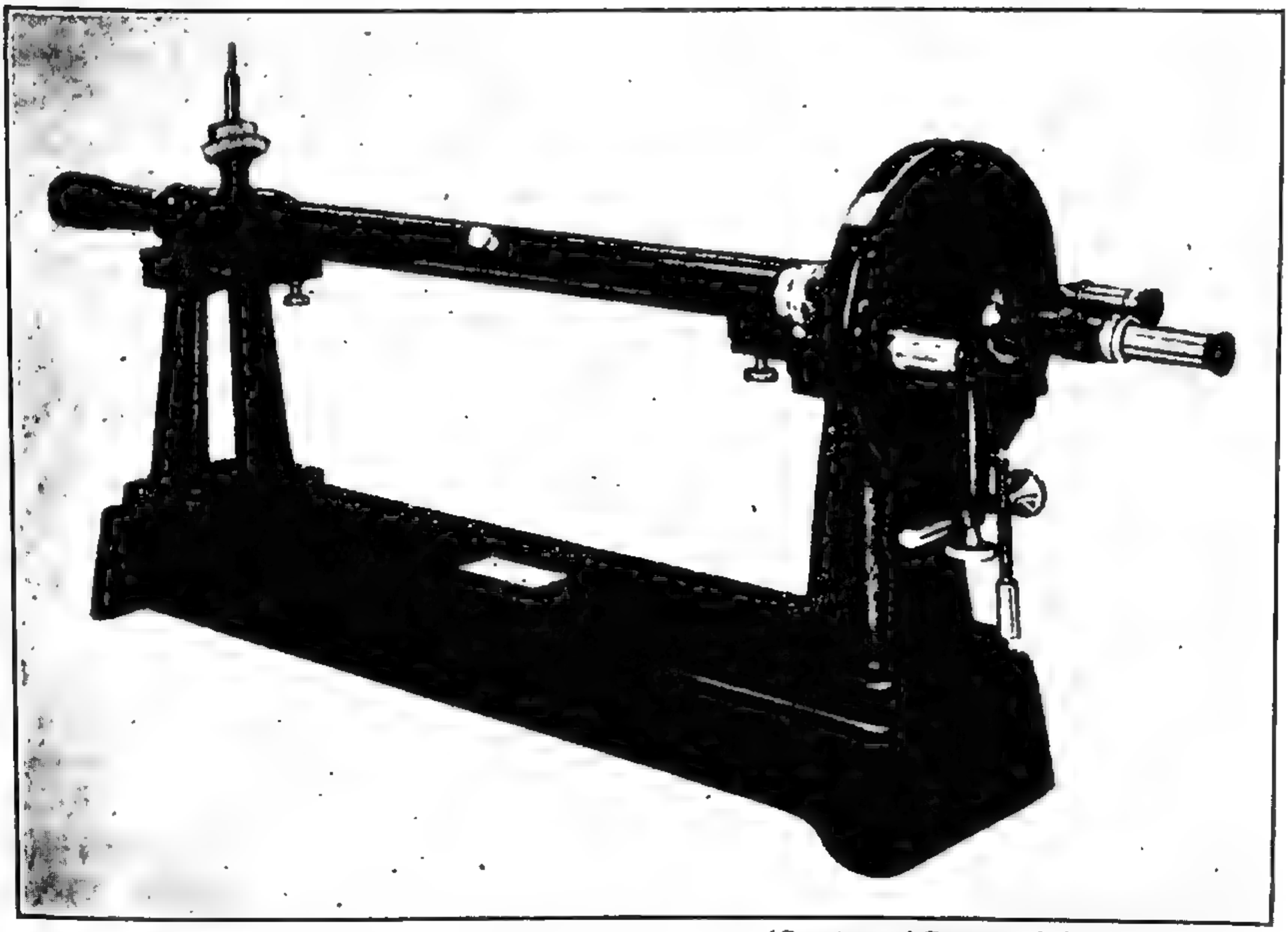
The graduations of the divided circle upon a polarimeter should be verified by taking check readings at different points upon opposite sides of the disk. The division and mounting of the circle in the best instruments are made with great accuracy, and, unless the disk has been warped or bent, check readings on opposite sides of the circle will agree much more closely than the observer can set the scale for a matched field.

Polariscope readings should always be verified upon the opposite scale. It is also well to reverse the circle 180° and repeat the



(*Courtesy of Akatos, Inc.*)

FIG. 107. Universal polarimeter of Lippich.



(*Courtesy of Gaertner Scientific Corp.*)

FIG. 107*a*. Gaertner polarimeter.

readings each way from the other side. By so doing the observer will have four sets of readings, the mean of which will practically eliminate all errors due to faulty scale division or eccentricity. The example given below of readings made upon a sugar solution will illustrate the method.

The adjustment of the half-shadow angle is made to the point of greatest sensibility, the angle being small for light-colored solutions and larger for dark liquids. Since altering the half shadow of the Lippich system produces a change in 0 point (p. 155), the adjusting lever should never be disturbed during a set of observations. The analyzer, if desired, can be brought back to the 0 of the scale for any change in the half-shadow angle by means of a small regulating screw (shown at *V*, Fig. 106). The better method, however, is to establish the 0 point upon the scale, as in the following example, and subtract this from the scale reading.

	0 Point		Sugar Solution		
	Right	Left	Right	Left	
Half-shadow angle = 6° Average	3.07	183.07	29.30	209.295	Temperature 20° C.
	3.09	183.085	29.28	209.28	
	3.11	183.11	29.295	209.29	
	3.08	183.075	29.27	209.28	
	3.10	183.10	29.285	209.29	
	3.09	183.088	29.286	209.287	
			3.090	183.088	
			26.196	26.199	
Reversing the circle 180°	183.075	3.08	209.270	29.265	Temperature 21° C.
	183.10	3.10	209.285	29.28	
	183.08	3.085	209.28	29.28	
	183.09	3.09	209.27	29.27	
	183.09	3.095	209.285	29.285	
	183.087	3.090	209.278	29.276	
			183.087	3.090	
			26.191	26.186	

Average of four readings, 26.193° for 20.5° C.

For a discussion of the light sources to be used with polarimeters the reader is referred to Chapter VII.

CHAPTER VI

THEORY AND DESCRIPTION OF SACCHARIMETERS

While the instruments described in the previous chapter are adapted to the examination of all optically active substances, saccharimeters are designed solely for polarizing sugars. For convenience the scale expressing angular rotation is replaced upon the saccharimeter by one graduated according to percentages.

The Quartz-Wedge Compensator

Owing to the many difficulties and inconveniences connected with the use of sodium or other monochromatic light in practical work, the French physicist Soleil[1] was led in 1845 to devise a means by which ordinary daylight or lamplight could be used for measuring the optical rotation of sugar solutions. This invention, known as the quartz-wedge compensation, is the characteristic feature of most saccharimeters.

In the quartz-wedge saccharimeter the polarizer and analyzer are both stationary; the rotation of the sugar solution is measured by shifting a wedge of optically active quartz between the solution and analyzer until the rotation of the wedge system at a certain thickness exactly neutralizes or compensates the rotation of the sugar solution. By means of a scale attached to the quartz wedge the rotation of the sugar in solution is measured in percentage.

The selection of quartz for compensation is based upon the fact that it has almost exactly the same rotation dispersion as cane sugar; i.e., a section of quartz and a cane-sugar solution of equal rotation for light of one wavelength will have very nearly equal rotations for light of all other wavelengths (see Table XXXI). The small disturbances due to the slight difference in rotation dispersion between sugars and quartz are eliminated by a bichromate light filter.

Single-Wedge System. The quartz wedges used in the construction of saccharimeters are cut perpendicularly to the optical axis of the quartz crystal; they may be either of dextrorotatory or levo-

[1] *Compt. rend.*, **20**, 1747 (1845); **21**, 426 (1845); **24**, 973 (1847); **26**, 162 (1848).

rotatory quartz, the method of mounting the wedge depending upon the character of the rotation. This can be seen more clearly by inspecting the following diagrams (Fig. 108).

In diagram I, *A* is a fixed plate of levorotatory quartz, and *B* and *C* two wedges of dextrorotatory quartz, of which *B* is movable and *C* stationary. The two wedges, which though of different size must have equal angular dimensions, may be considered to form together a single section with sides parallel to the plate *A* and perpendicular to the axis of light through the instrument. The thickness of the two wedge sections can be increased or diminished by moving wedge *B* to the right or left. At the 0 point of the instrument the right rotation of the section *lmno* of the wedges *B* and *C* exactly neutralizes the left

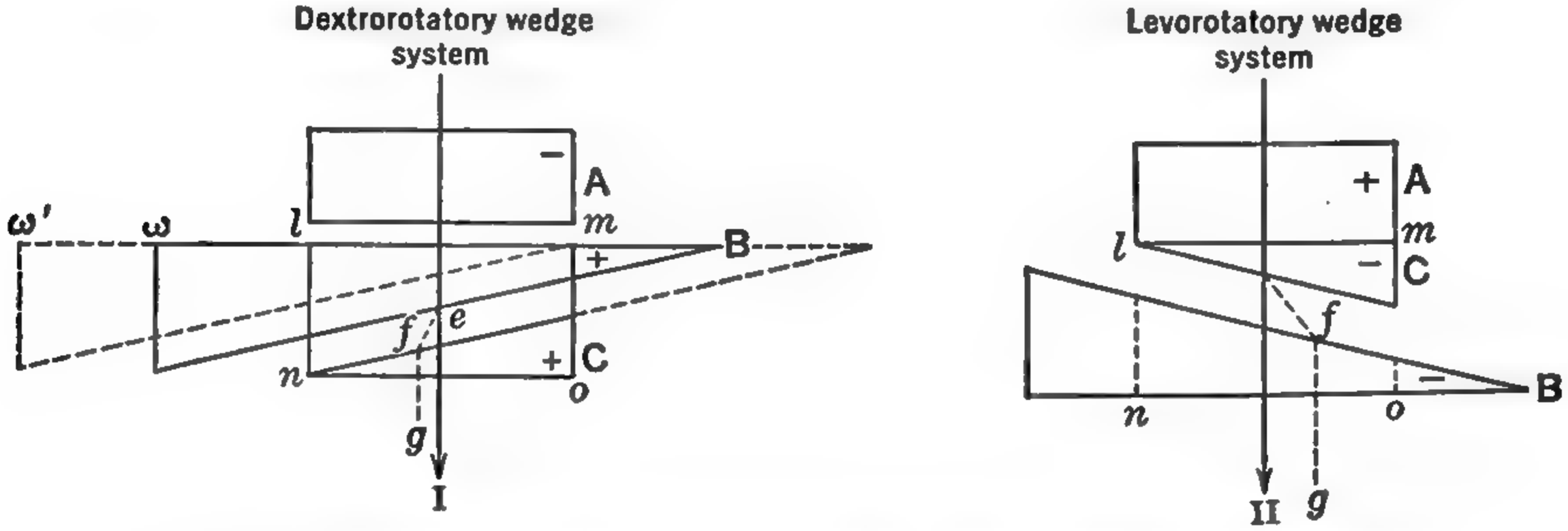


FIG. 108. Showing construction of single-quartz-wedge compensation.

rotation of the quartz plate *A*. If a tube of dextrorotatory sugar solution is now placed in the instrument between the polarizer and the compensation plate *A*, the optical neutrality is destroyed, and it will be necessary to decrease the thickness of the two-wedge section by sliding *B* from ω towards ω' until the excess of left rotation in *A* over *B* and *C* exactly neutralizes the right rotation of the sugar solution. If the solution of sugar is left-rotating, it will be necessary to slide *B* in the opposite direction until the excess of right rotation in *B* and *C* over *A* equals the left rotation of the sugar. In a levorotatory wedge system (diagram II) the compensation plate *A* is dextrorotatory and the wedges *B* and *C* levorotatory, the compensating motion of wedge *B* being the reverse of that in diagram I.

Owing to the lateral refraction of light from the inclined surfaces of the wedges through the intervening air space (as shown by the dotted line *efg*), the planes of quartz are separated only just sufficiently to allow free movement of the parts without friction. The circumstance that the field is not exactly at the end point, when the thickness of the two-wedge section agrees with that of the compensating plate,

is due to this lateral refraction. The shifting of 0 point due to refraction depends upon the wavelength of light; the difference in 0 point between red light of 760-mμ wavelength and violet light of 396.8-mμ wavelength was found by Schönrock to be 0.059° for the Ventzke sugar scale.

The scale of the saccharimeter is attached to the large or movable wedge, and is read by means of a vernier scale attached to a regulating screw. If the zero marks of the two scales do not agree when the two halves of the field correspond in shade, they can be brought into coincidence by shifting the vernier slightly to the right or left by means of a key which fits the regulating screw. The vernier is never to be moved except for making this adjustment, and when the two scales are once set it rarely has to be disturbed. Owing to the inevitable slight fluctuations in the 0 point of saccharimeters, it is best to correct the reading by the 0-point error and not to adjust the scale unless there is a persistent difference of the 0 point in one direction greater than 0.1°. The method of reading the saccharimeter scale can be seen from Figs. 110 and 111.

Double-Wedge System. An elaboration of the quartz-wedge system just described is the double-wedge compensation introduced by Schmidt and Haensch. The arrangement of the parts in the double-wedge system is shown in Fig. 109.

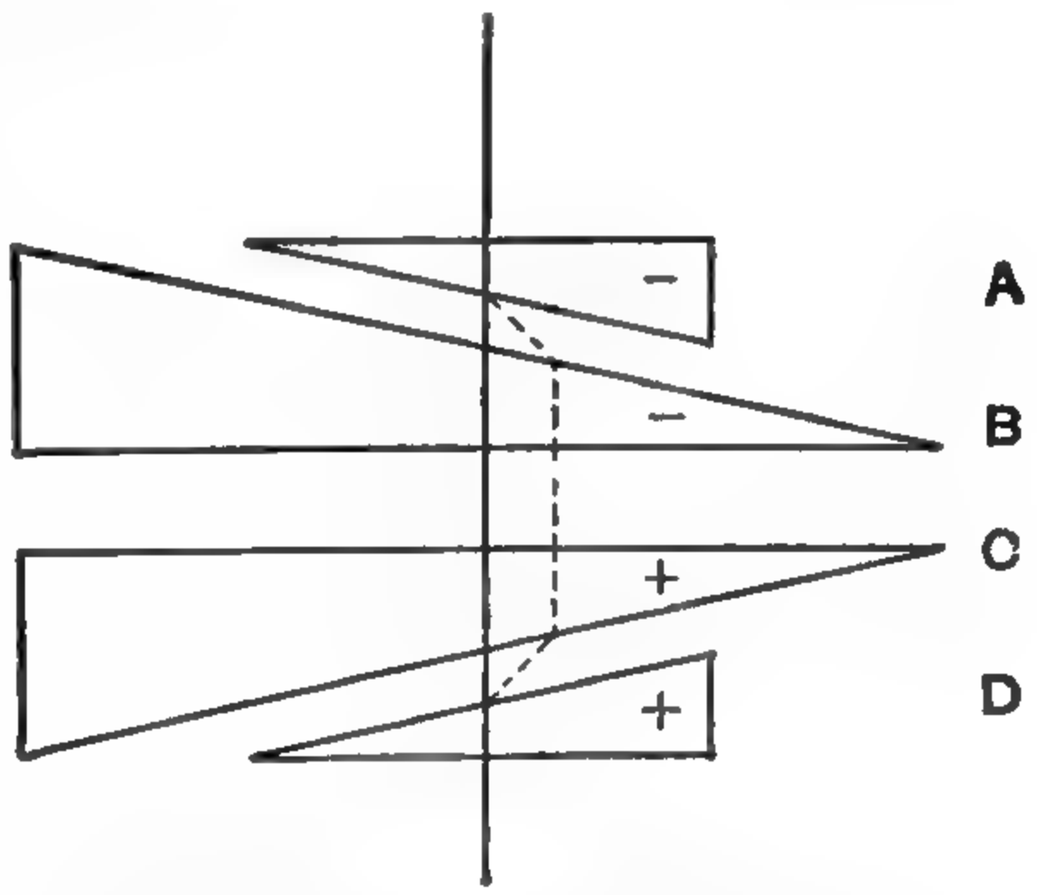


Fig. 109. Showing construction of double-quartz-wedge compensation.

In the double-wedge system the compensation plate is lacking, this being supplied by one or the other of the pair of wedges, which are of opposite rotation. The smaller wedges A and D are stationary, and the larger wedges B and C movable. B and C are usually mounted with their points in the same direction in order to equalize the refraction of the light rays in the air spaces between the inclined surfaces of quartz (as indicated by the dotted line); for this reason also the corresponding wedges of each system are made as near alike as possible. Each of the large wedges is provided with a scale. These may be read through the same telescope as upon the Schmidt and Haensch saccharimeter (Fig. 110), or by separate telescopes as in the Frič instruments (Fig. 111).

In using the double-wedge system for dextrorotatory substances, the scale K (Fig. 110) is set at 0 with its vernier, and the optical rota-

tion measured upon the scale *A*; for levorotatory solutions, *A* is set at 0 and the scale *K* employed. An additional advantage of the double-wedge system consists in the fact that any reading obtained upon

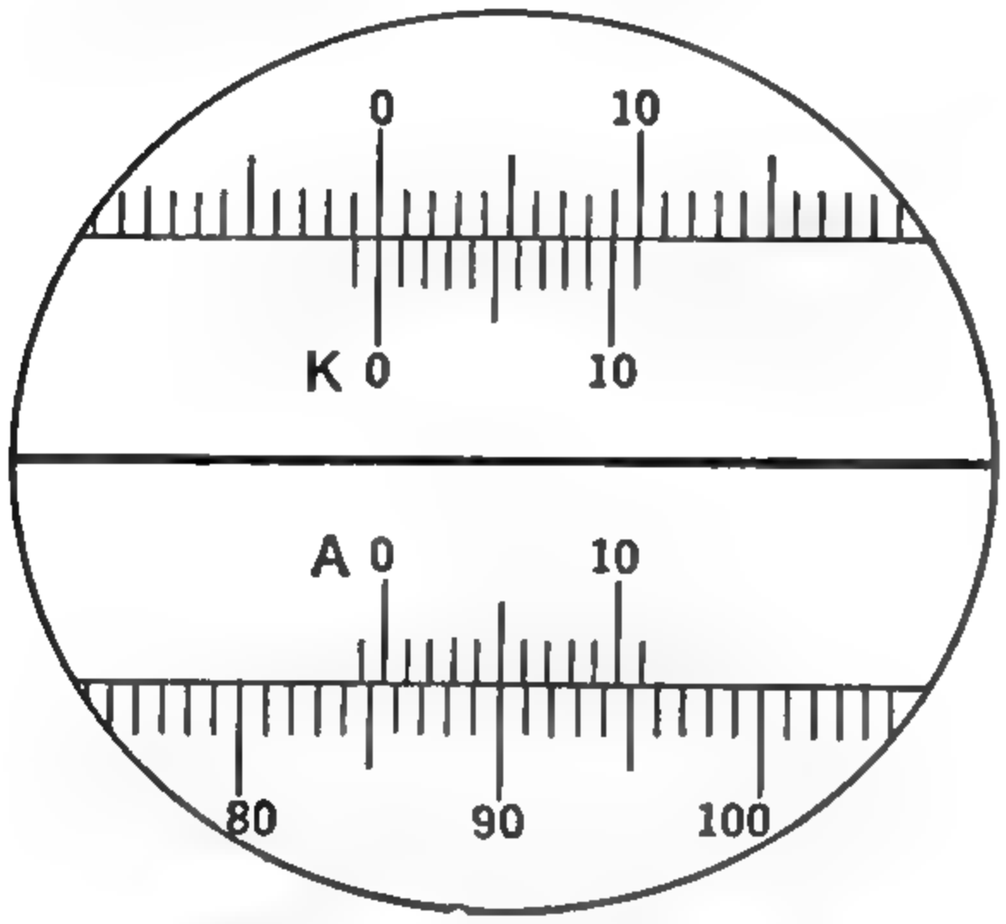


FIG. 110. Scale of double-wedge Schmidt and Haensch saccharimeter.

K, control scale; *A*, working scale indicating 85.5° Ventzke.

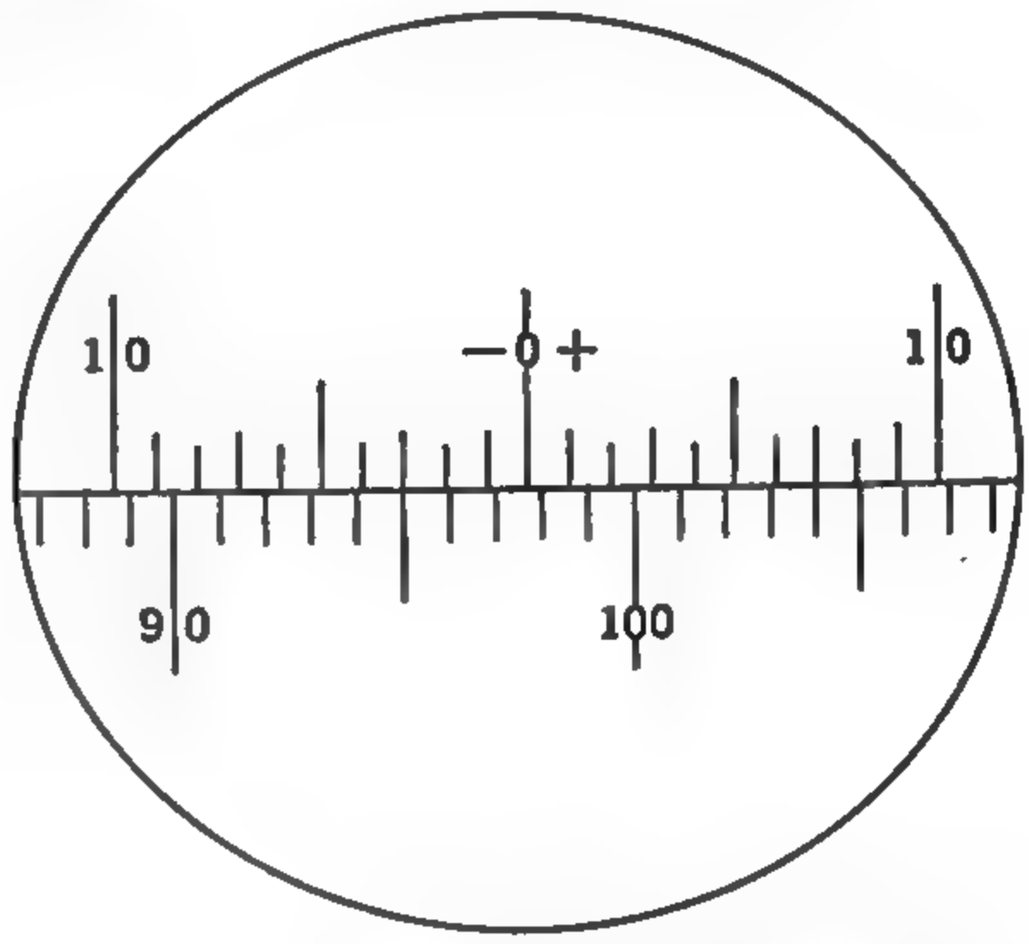


FIG. 111. Scale of Fric saccharimeter with double vernier indicating 97.7° Ventzke. (The division between scale and vernier is intensified; in reality no dividing line is seen.)

the working wedge can be immediately verified by removing the tube of solution and moving the control wedge to the point of compensation. The control wedge under such conditions gives the true reading directly, even though the working wedge has a zero-point correction.

Zero-Point Determination			Polarization of Mat Sugar		
Control-Wedge Scale	Working-Wedge Scale	Difference	Control-Wedge Scale	Working-Wedge Scale	Difference
0.00	0.10	+0.10	0.00	89.40	89.40
11.55	11.65	+0.10	0.75	90.15	89.40
20.75	20.80	+0.05	2.15	91.50	89.35
32.20	32.30	+0.10	2.90	92.30	89.40
43.75	43.80	+0.05	3.85	93.25	89.40
52.50	52.55	+0.05	5.45	94.85	89.40
61.85	61.95	+0.10	6.55	96.00	89.45
70.50	70.60	+0.10	7.95	97.30	89.35
81.15	81.30	+0.15	9.10	98.45	89.35
91.15	91.25	+0.10	10.15	99.55	89.40
Average zero point		+0.09	Average polarization uncorrected		89.39
			Zero-point correction =		0.09
			Corrected polarization =		89.30

Zero-Point Determination. The zero-point correction of the working wedge can be determined very accurately by taking check readings at different parts of the scale upon the control. By making polarizations in the same way, the local defects of scale or wedge will be almost wholly eliminated. The readings are then made without removing the tube, the difference between the two scales being the *uncorrected* polarization. The preceding table, giving the readings upon the working-wedge scale for various positions of the control, will illustrate the method.

The Sugar Scale and Normal Weight of Saccharimeters

The 100° point of a saccharimeter scale is usually based upon the rotation of a definite weight (the so-called normal weight) of chemically pure sucrose dissolved in water to 100 ml. at a specified temperature and polarized at the same temperature in a 200-mm. tube. The greatest confusion has prevailed in saccharimetry in the past, and unfortunately still prevails, not only as to the size of the normal weight of sugar to be taken for a specified scale, but also as to the conditions of volume and temperature under which this normal weight is to be polarized.

French Sugar Scale. The 100° point of the sugar scale employed upon saccharimeters of French manufacture was originally based upon the rotation in sodium light of a plate of dextrorotatory quartz 1 mm. in thickness and cut exactly perpendicular to the optical axis. The choice of quartz as a standard proved to be unfortunate, for, owing either to mistakes of polarimetric measurement or to defects in the quartz (through natural imperfection or mistakes in cutting), the rotation of the 1-mm. plate has been given a different value from time to time, the results ranging from +20.98°, the early figure of Biot, to 22.67°. Landolt[2] gives the figure 21.723 as the average of the closely agreeing results of four different observers for spectrally pure sodium light at 20° C. Lowry[3] found 21.7283° at wavelength 589.25 mμ, but later Einsporn and Schönrock[4] reported 21.724°.

Since the optical rotation can be measured with greater precision than the thickness of a 1-mm. quartz plate, it was finally decided to abandon the latter as the primary standard, and to adopt, for fixing the 100 point of the French saccharimeters, a rotation of 21° 40′, or 21.667°, as given by Broch in 1852.

[2] "Das optische Drehungsvermögen," 2nd ed., p. 374, 1898.
[3] *Phil. Trans.*, 212, 288 (1912/13).
[4] *Z. Ver. deut. Zucker-Ind.*, 89, 1 (1939).

The number of grams sucrose which, dissolved to a total volume of 100 ml. at 20° C., gives the same rotation as either standard, has necessarily varied also. The values obtained in the earlier work, based on 1 mm. of quartz, ranged from 16.000 g. (Dubrunfaut) to 16.471 g. (Clerget and Biot).

For a long time a normal weight of 16.35 g. was used in technical work with the Soleil-Duboscq saccharimeter. In 1875 the value of Girard and de Luynes, 16.19 g., was adopted as the official weight and remained such for more than 20 years, notwithstanding the severest criticism. Sidersky, in 1885, reported the figure 16.29 g., and this was accepted 11 years later by the International Congress of Applied Chemistry at Paris. The French Ministry of Finance commissioned Mascart and Bénard to check this figure, and these investigators reported[5] 16.284 g., in terms of mass, to give a rotation of 21.67°, but through an error the corresponding weight in air was stated to be 16.291 g., and 16.29 g. became the official weight in France. Pellat[6] found 16.285 g. (weight in vacuo), closely agreeing with Mascart and Bénard. The new official weight was criticized, as being too high, by Saillard and others. At Saillard's request, Bates and Phelps took up the problem anew;[7] they calculated the value of the French normal weight from the simple relation between the standard value of 21.667° for the circular rotation, and the rotation 34.620° for the 26-g. normal weight. If the difference in the specific rotation at the two concentrations is disregarded, the French normal weight would equal $\frac{26.000 \times 21.667}{34.620}$, or 16.272 g. This result must be corrected for change in specific rotation by multiplying by the specific rotation for 26 g. (66.54),[8] and dividing by that for 16 g. (66.552°). The final result for the French normal weight is thus 16.269 g., weighed in air with brass weights, and dissolved to 100 ml. at 20° C. This normal weight was adopted officially by the French government in 1938.

According to Jobin[9] the principal manufacturers of polarimeters and saccharimeters in France calibrate the sugar scale on circular instruments so that 16.29 g. sucrose give a reading of 100, on the basis of 66.5° for the specific rotation of sucrose. If the 16.29 g. is the

[5] *Ann. chim. phys.*, [7], 17, 125 (1899).

[6] *Ann. chim. phys.*, [7], 23, 289 (1901).

[7] *Bur. Standards J. Research*, 17, 347 (1936).

[8] Calculated from Schönrock's formula given in Geiger's *Handbuch der Physik*, 19, 705–776 (1928). If the formula given by Schönrock in Kohlrausch's "Praktische Physik," 17th ed., 1935, is used, the result for the French normal weight is the same, 16.269 g.

[9] *Facts About Sugar*, 12, 352 (1921).

weight reduced to vacuo, the rotation for the 100 point of the sugar scale is $2 \times 66.5 \times 16.29/100$, or 21.666°. The thickness of the normal quartz plate is given by Jobin as 0.9976 mm., and the rotation of the 1-mm. quartz plate as 21.7182°. This also gives a rotation of 21.666° for the normal quartz plate. It appears therefore, that, in spite of errors in Jobin's basic figures, both polarimeters and saccharimeters are calibrated in close agreement with the standard rotation of 21.667°.

Ventzke or German Sugar Scale. The sugar scale most generally used outside of France and the one employed upon all German saccharimeters is that of Ventzke. This scale as originally devised by Ventzke[10] was based upon the rotation of a solution containing 25 per cent sucrose by weight, but this was later changed to that of a solution of 1.1 sp. gr. $^{17.5°}_{17.5°}$ read in a tube 234 mm. long. However, it was soon found inconvenient as well as inaccurate to make the specific gravity of a solution the basis for saccharimetric work, and the number of grams of sugar contained in a solution of 1.1 specific gravity was introduced as the normal weight; this weight was determined to be 26.048 g. weighed in air with brass weights and dissolved at 17.5° to 100 ml. The length of the tube was reduced to 200 mm.

Mohr Cubic Centimeter Standard. With the introduction in 1855 of the Mohr[11] cubic centimeter (the volume of 1 g. of water at 17.5° C. weighed in the air with brass weights), the original normal weight of 26.048 g., designed for metric cubic centimeters, was strangely enough retained and used for determining the 100° point of the sugar scale. In this way the standard was established which up to 1900 was the only one recognized for the Ventzke scale. It was officially used in Java until 1930, and in some places has not been entirely abandoned yet. In accordance with this standard, the 100° point of the sugar scale is obtained by dissolving 26.048 g. of chemically pure sucrose (weighed in air with brass weights) in 100 Mohr cc. at 17.5° C. and polarizing the same in a 200-mm. tube at 17.5° C. in a saccharimeter whose quartz-wedge compensation has also a temperature of 17.5° C. This normal weight calculated to 100 ml. (volume of 100 g. water at 4° C.) is equal to 26.048 g. ÷ 1.00234 = 25.9872 g. (1 Mohr cc. = 1.00234 ml.).

Milliliter Standard. On account of the confusion and mistakes resulting from two standards of volume, the International Sugar Commission, at its third meeting in Paris, 1900, advocated the abandonment of the Mohr for the metric cubic centimeter (milliliter), and

[10] *J. prakt. Chem.*, 25, 84 (1842); 28, 111 (1843).
[11] "Chemisch-analytische Titrirmethode," pp. 44–50, 1886.

in so doing also recommended that the temperature of polarization be made 20° C. The change in temperature from 17.5° C. to 20° C. necessitated a recalculation of the normal weight owing to the difference in specific rotation of cane sugar and quartz at these two temperatures. The calculation is made by the following equation, in which 0.000184 is the coefficient of decrease in specific rotation of sucrose at 20° C., 0.000148 the coefficient of increase in rotation due to the effect of temperature upon wedge and scale, and 0.000008 the coefficient for expansion of the glass observation tube:

$$\frac{26.048}{1.00234}\{1 + (0.000184 + 0.000148 - 0.000008) \quad (20° - 17.5°)\}$$
$$= 26.0082 \text{ g.}$$

The International Commission decided, however, to make the new normal weight exactly 26 g., and in accordance with its recommendation the following definition for the 100° point of the Ventzke sugar scale was adopted: "The 100° point of the saccharimeter scale is obtained by polarizing a solution containing 26.000 g. of pure sucrose (weighed in air with brass weights) in 100 ml. at 20° C. in a 200-mm. tube in a saccharimeter whose quartz-wedge compensation must also have a temperature of 20° C."

But, according to a statement made in private conversation by Mr. Haensch, in 1930, Schmidt and Haensch never made a change in the scale of their saccharimeters up to that time, and it is doubtful that other manufacturers did.

At the Seventh Session of the International Commission for Uniform Methods of Sugar Analysis, in 1912, Bates reported that the 100 point of the German saccharimeters was not quite correct, and a committee was appointed to re-examine it. Bates and Jackson[12] found that the normal sugar solution prepared according to the directions adopted in 1900 read 99.895° on the German sugar scale. This value was criticized by Herzfeld,[13] but some later investigators reported even lower figures. Kraisy and Traegel,[14] of the German Sugar Institute, obtained an average of 99.834°. Staněk,[15] at the Research Institute of the Czechoslovakian Sugar Industry, found figures ranging from 99.81° to 99.90° for sucrose purified in different ways. The polarization decreased with repeated precipitations by alcohol. Browne and Zerban,[16] as the result of two independent de-

[12] *U. S. Bur. Standards, Sci. Paper* 268, 1916.
[13] *Z. Ver. deut. Zucker-Ind.*, **67**, 407 (1917).
[14] *Z. Ver. deut. Zucker-Ind.*, **74**, 193 (1924).
[15] *Z. Zuckerind. čechoslovak. Rep.*, **45**, 417, 425 (1920/21).
[16] *J. Assoc. Official Agr. Chem.*, **11**, 106 (1929).

terminations, reported the values 99.907° and 99.912°, averaging 99.91°.

At the Eighth Session of the International Commission in 1932 Bates gave the average of all these results as 99.90°, and this figure was adopted as the standard. It was recommended that the change to the new standard be effected either by changing the scale and using the normal weight of 26.000 g., or by using the old scale and changing the normal weight to 26.026 g. Following a suggestion by Browne and Balch,[17] this normal weight, and the corresponding half normal weight of 13.013 g., are made in hexagonal form so that they may be readily distinguished from the cylindrical weight of 26.048 g. and the cubical weight of 26.000 g. The manufacturers of saccharimeters have since then decided to equip new instruments with the new scale. When purchasing a saccharimeter, the chemist must make sure of the scale employed, so that he may use the correct normal weight.

The International Commission also recommended that the new scale be known as the International Sugar Scale, and that the degrees on the scale be expressed as degrees Sugar or degrees S., to avoid confusion with degrees Ventzke or degrees V., found on the previous scale of Schönrock (p. 180). The new scale, however, is not used internationally[18] by any means, since many countries employ either the French scale or the bidecimal, 20-g. scale (p. 180). It does not really matter what scale is used, as long as the normal weight solution gives a reading of 100.

The 26 g. normal weight in combination with the Ventzke scale is still widely used in control and regulatory work for the polarization of raw sugars and other impure sugar products requiring clarification; the reason for this is explained on pages 334–336. The old system is also quite generally retained in the polariscopic analysis of complex sugar products, such as for instance those containing commercial glucose, where the scale error is of no practical significance; if desired, a correction for this error can be readily applied by using either

[17] *Ind. Eng. Chem., Anal. Ed.*, 5, 283 (1933).

[18] Further confusion in the use of the word international is due to the fact that the term was previously applied by Sidersky and Pellet to the 20-g. or bidecimal normal weight in 1896 at the Second International Congress of Applied Chemistry and this designation was employed in the discussions of the third, fourth and fifth meetings of the Congress. Until complete international agreement is reached upon the choice of a normal weight the term international is to be avoided as misleading. The introduction of a new scale differing by only 0.1° from the old Ventzke scale was unfortunate because of the liability of error from confusion in the use of slightly differing normal weights.

of the two procedures recommended by the International Commission.

United States Coast Survey Standard. The old standard of the Ventzke scale was the one adopted by the Department of Weights and Measures of the United States Coast and Geodetic Survey, and was employed for many years by the United States Treasury Department in the Custom House laboratories. The 100° point of the scale was taken as the polarization of 26.048 g. (in vacuo) of pure sucrose dissolved to 100 ml. of solution at 17.5° C. and polarized at this temperature in a 200-mm. tube. To avoid the labor of reducing this weight of sugar to vacuo, the flasks employed for the Coast Survey standard were graduated to contain 100.06 ml., the excess of 0.06 ml. being taken to correct the error of weighing the sugar in air against brass weights. These flasks contain 0.174 ml. less than the old Mohr cubic centimeter flasks (100.234 ml.), which difference, unless compensated, would cause the normal weight of 26.048 g. of pure sucrose to polarize 0.17° V. too high. To save the operators the trouble of making this correction, the correction of 0.17 was applied to the quartz test plates used for controlling the instruments. The computed values of the Coast Survey test plates were thus 0.17° V. lower than the values marked by the instrument makers for the Mohr cubic centimeter standard.

The policy of the Coast Survey in adopting a standard different from that in current use gave rise to much confusion. According to the work of both Sawyer[19] and Rolfe,[20] there were many old instruments in the United States, which were standardized for a normal weight of 26.048 g. in 100 ml.

Value of German Scale in Circular Degrees. The rotation value for quartz corresponding to the 100° point of the scale fixed in 1900 for the 26-g. weight was measured by Schönrock,[21] who found it to equal 34.657 circular degrees for spectrally pure sodium light, at 20° C. According to Bates and Jackson (p. 178) the rotation of the normal quartz plate, measured under the same conditions, is 34.620 circular degrees. This value was adopted at the Eighth Session of the International Commission in 1932.

The Bidecimal Sugar Scale. Sidersky and Pellet[22] proposed in 1896 the adoption of a so-called international sugar scale, based upon a normal weight of 20 g. This project was supported by Dupont[23] and

[19] *J. Am. Chem. Soc.*, **26**, 990 (1904).
[20] *Technology Quarterly*, **18**, 294 (1905).
[21] *Z. Ver. deut. Zucker-Ind.*, **54**, 521 (1904).
[22] *Intern. Congr. App. Chem.*, **2**, 379, 391, 514 (1896); **4**, 229.
[23] *Intern. Congr. App. Chem.*, **3**, 307, 451 (1900); **3**, 129 (1903).

later by Browne,[24] Deerr,[25] Bryan,[26] and others. Among the advantages claimed for the proposed 20-g. scale are the following: It is a compromise between the French and German scales; calculations are simplified by use of a decimal weight; aliquots of 50, 25, 20, 10, and 5 ml. of the 100 ml. solution contain even gram quantities; the normal weight, its fractions, and its multiples are always available as one-piece units.

These advantages have led many chemists to adopt the 20-g. scale, and instruments so equipped are extensively used in Australia, Fiji, Egypt, Mauritius, and other countries.

The rotation for the 100 point used by French manufacturers of instruments with this scale is 26.600 circular degrees. This rotation was calculated by multiplying the standard rotation for the French scale, 21.667°, by 20, and dividing by 16.29, representing the old official French normal weight. This could be done because the specific rotation of sucrose at the two concentrations differs by only 0.001, well within the limits of error. Baissac[27] has pointed out that, if the normal weight for the French scale must be changed to 16.269 g. (p. 176), that for the 20-g. scale must be proportionately reduced to 19.973 g. The advantages of a decimal normal weight will thus be lost, unless the 100 point of the scale is raised to 26.636 circular degrees.

Bichromate Light Filter. Schönrock[28] has shown that in establishing the 100° point of the Ventzke scale by means of sucrose the white light must be filtered through a 1.5-cm. layer of 6 per cent potassium bichromate solution in order to eliminate the errors of rotation dispersion between cane sugar and quartz produced by the light of shorter wavelength at the violet end of the spectrum. This light filter has been adopted by the Physikalisch-Technische Reichsanstalt of Germany and also by the National Bureau of Standards[29] in defining the 100° point of the saccharimeter scale, and its use is imperative for all accurate work. Many saccharimeters have a 3-cm. cell, and for this length of liquid a 3 per cent bichromate solution is sufficient (centimeter length of cell × percentage bichromate = 9). For

[24] *J. Ind. Eng. Chem.*, **10**, 916 (1918).
[25] *Louisiana Planter*, **62**, 282 (1919).
[26] *J. Assoc. Official Agr. Chem.*, **4**, 324 (1920/21).
[27] *Rev. agr. Maurice*, **1937**, 120.
[28] *Z. Ver. deut. Zucker-Ind.*, **54**, 521 (1904).
[29] The International Commission has recommended that "the polarization of the normal solution (26.000 g. of pure sucrose dissolved in 100 ml., and polarized at 20° C. in a 200-mm. tube, using white light and the dichromate filter as defined by the Commission) be accepted as the basis of calibration of the 100° point on the International Sugar Scale."

carbohydrate materials of greater rotation dispersion than cane sugar, such as dextrin and commercial glucose, it has been found necessary to use a solution of double the above concentration (centimeter length of cell × percentage bichromate = 18) in order to secure constancy of results among different observers for different sources of white light; this constancy is only approximate, however, and not absolute.

In this connection it is important to note that the rotations of the normal weight of sucrose with bichromate-filtered white light and with sodium light, though very closely agreeing, are not absolutely identical owing to the slight differences in optical center of gravity. Measurements by Schönrock[30] show that, while a normal sugar solution at 20°C. for bichromate-filtered white light is exactly equal to the rotation of a quartz plate of 100° V. (34.657 angular degrees), by using sodium light a quartz plate of 100.03° V. (34.667 angular degrees) would be required.[31] The relationship between Ventzke degrees for bichromate-filtered white light and monochromatic light of different wavelengths is seen from Table XXXI.[32]

TABLE XXXI

ROTATION OF QUARTZ AND SUCROSE FOR DIFFERENT KINDS OF LIGHT

Source of Light	Mean Wavelength mμ	Angular Rotation, 20° C.		Degrees Ventzke
		Quartz Plate (1.595 mm.)	Sucrose Solution (26 g. in 100 ml. in 200-mm. Tube)	
White light filtered through 1.5 cm. of bichromate solution, about	600	34.65	34.65	100.00
Spectral pure sodium light	589.3	34.657	34.667	100.03
White light, Welsbach, unfiltered, about	551	39.82	39.87	100.12
Yellow-green mercury	546.1	40.73	40.81	100.19
Green tantalum	535	42.49	42.67	100.42
Blue strontium	460.7	58.65	59.18	100.91
Violet rubidium	420.2	71.78	72.87	101.52

[30] *Z. Ver. deut. Zucker-Ind.*, **54**, 521 (1904).

[31] Even the source of sodium light affects the rotation. According to Einsporn and Schönrock (*Z. Ver. deut Zucker-Ind.*, **89**, 1 [1939]), 100 sugar degrees equals 34.620 circular degrees with sodium light obtained by volatilizing soda sticks in the Linnemann gas-oxygen blast lamp, but 34.613 circular degrees with light from an electric sodium-vapor lamp (wavelength 589.3 mμ).

[32] Compiled from results by Landolt and by Schönrock.

It is seen that, while the quartz and sugar exactly agree for bichromate-filtered light, the sugar is rotated to a continually greater extent than quartz for light of decreasing wavelength. The normal sugar solution, reading 100° V. with filtered white light, was found to read 100.12° with unfiltered white light. The eyes of some observers are more sensitive than those of others to the disturbances of rotation dispersion when unfiltered light is used (owing perhaps to some difference in the pigment of the eye), so that for accuracy and constancy of results in all saccharimetric measurements the bichromate filter should never be omitted.[33]

It has already been noted (p. 178) that values differing from those of Table XXXI have been reported by Bates and Jackson, and by Kraisy and Traegel. The results obtained by these investigators are summarized as follows:

	Bates and Jackson	Kraisy and Traegel
Thickness of normal quartz plate	1.5934 mm.	1.5921 mm.
Rotation of normal quartz plate at 589.25 mμ	34.620°	34.599°
Rotation of normal sugar solution at 589.25 mμ	34.617°	34.609°
Rotation of normal quartz plate at 546.1 mμ	40.690°	40.660°
Rotation of normal sugar solution at 546.1 mμ	40.763°	40.738°

The figures obtained by Kraisy and Traegel are in every case lower than those of Bates and Jackson, which in turn are lower than those of Herzfeld and Schönrock. When the values for the rotation of the normal sugar solution in spectrally pure sodium light are converted into specific rotation, the results of Bates and Jackson and of Kraisy and Traegel are found to agree much more closely with accepted values than those of Herzfeld and Schönrock. It is also interesting to note that, whereas Schönrock found the normal sugar solution to read higher with sodium light than the normal quartz plate, Bates and Jackson found a lower figure; Kraisy and Traegel report a higher value, but they calculated the rotation of the quartz plate from that of the sugar solution by means of the relationship found by Schönrock.

[33] At its New York Meeting (Sept. 10, 1912) the International Commission adopted the following resolution: "Wherever white light is used in polarimetric determinations, the same must be filtered through a solution of potassium bichromate of such a concentration that the percentage content of the solution multiplied by the length of the column of the solution in centimeters is equal to nine."

Graduation of Saccharimeter Scales. Manufacturers of saccharimeters in establishing the 100° point of their sugar scales employ a carefully standardized quartz plate instead of the normal weight of sucrose. The errors and inconveniences incident to the preparation of chemically pure sucrose and to making the solution up to exact volume are thus avoided; the plate, moreover, has the advantage of being a standard which at constant temperature is always unchangeable as long as it is protected from mechanical shock. Messrs. Schmidt and Haensch[34] thus describe the method of graduating the scales of their saccharimeters:

The establishment of the scale divisions of our saccharimeters is made at a *temperature of 20° C.* After fixing the 0 point the linear distance of the 100° division is determined by means of a normal quartz plate reading exactly 100° and standardized at the Physikalisch-Technische Reichsanstalt. This linear distance is then divided into 100 exactly equal parts, the intermediary divisions being also verified by means of corresponding normal standardized quartz plates. The surfaces of the quartz wedges are made perfectly plane so that a quartz stratum of half thickness corresponds to a half value in the division. Slight errors cannot be prevented, as it is impossible to obtain quartz wedges of the necessary length which are absolutely optically homogeneous throughout. The variableness in the specific rotation of sucrose with concentration of solution is not taken into consideration in the establishment of the scale division, and this must be corrected for by calculation. Aberrations in the scale division caused by impurities in the quartz can be detected by the control observation tube.

The view that the Ventzke scale of modern saccharimeters is corrected for variations in specific rotation of sucrose with concentration, either by curving the surface of the quartz wedges or by unequal spacing of the scale divisions, is not substantiated by the above statement.

Effect of Concentration upon Scale Reading. A table has been calculated by Schmitz[35] to correct for the changes in specific rotation of sucrose through varying concentration, which gives the actual sucrose value of each scale division of the saccharimeter. These corrections, which were calculated by Schmitz's formula, $[\alpha]_D = 66.514 - 0.0084153\,c$, would seem in light of more recent work to require considerable modification. The formula of Landolt,

$$[\alpha]_D^{20^\circ} = 66.435 + 0.00870\,c - 0.000235\,c^2, \quad (c = 0 \text{ to } 65)$$

calculated from the combined observations of Tollens, and of Nasini and Villavecchia, is regarded as more accurate (see p. 268). In

[34] In a private letter.

[35] Ber., **10**, 1414 (1877); *Z. Ver. deut. Zucker-Ind.*, **28**, 63, 887 (1878).

Table XXXII the sucrose values of the Ventzke scale for different concentrations have been recalculated by Landolt's formula. The later formula of Schönrock gives the same results as Landolt's. The values of Schmitz are also shown for comparison.

TABLE XXXII

EFFECT OF CONCENTRATION OF SUCROSE UPON SACCHARIMETER READINGS

Scale Division	Concentration Grams Sucrose, 100 ml., 20° C.	Specific Rotation Sucrose, 20° C.	Actual Sucrose Value of Scale Division	
			By Landolt's Formula	By Schmitz's Formula
100.00	26.00	66.502	100.00	100.00
96.00	24.96	66.506	96.00	95.98
95.00	24.70	66.507	94.99	94.98
90.00	23.40	66.510	89.99	89.97
85.00	22.10	66.513	84.99	84.96
80.00	20.80	66.514	79.99	79.95
75.00	19.50	66.515	74.99	74.94
70.00	18.20	66.516	69.99	69.93
65.00	16.90	66.515	64.99	64.92
60.00	15.60	66.514	59.99	59.92
55.00	14.30	66.511	54.99	54.92
51.00	13.26	66.509	50.99	50.92
50.00	13.00	66.508	50.00	49.92
45.00	11.70	66.505	45.00	44.92
40.00	10.40	66.500	40.00	39.92
35.00	9.10	66.495	35.00	34.92
33.00	8.58	66.492	33.00	32.93
32.00	8.32	66.491	32.01	31.93
30.00	7.80	66.489	30.01	29.93
25.00	6.50	66.481	25.01	24.94
20.00	5.20	66.474	20.01	19.95
15.00	3.90	66.465	15.01	14.96
10.00	2.60	66.456	10.01	9.97
6.00	1.56	66.443	6.01	5.98
5.00	1.30	66.442	5.00	4.98

It will be seen from this table that the greatest deviation of the actual sucrose value from its scale division according to Landolt's equation is only 0.01° V. (or S.), which is too small to be detected by the ordinary saccharimeter. The maximum error according to Schmitz is 0.08° V. (or S.).

As regards the concentration of sucrose employed in ordinary saccharimetric work, the variations due to changes in specific rotation may therefore be safely disregarded. The small extent of these variations, which are distributed both above and below the scale division, justifies the policy of the manufacturers in neglecting this factor when establishing the divisions of the saccharimetric scale.

Verification of Scales of Saccharimeters

On account of the optical imperfections which quartz wedges occasionally possess, it is important that every user of a saccharimeter should verify the accuracy of his instrument.

Owing to the fact that the quartz parts of the saccharimeter are mounted close to the objective of the telescope, the very local imperfections of the wedge system are fortunately unnoticed, since, when the telescope is focused upon the polarizer, the cone of light rays emanating from the different parts of the field covers an area of the compensator equal to the aperture of the analyzer diaphragm (about 6-mm. diameter) and thus distributes and neutralizes any slight local errors due to defects of the quartz. Such defects in the fixed part of the system (small wedge and compensation plate) are of no account, since the rotatory power of this remains constant; the predominant optical defects of the large movable wedge are the only ones which vitiate the results of observation.

Since local optical impurities in the large wedge are diffused over a considerable area, for the reason given above, the errors in the saccharimeter scale never consist of sudden jumps, but only of gradual undulations. It is unnecessary, therefore, as Landolt has shown, to standardize every division of the scale. The errors at every fifth degree, if plotted upon coordinate paper, are sufficient to establish a correction curve from which the error of any division upon the scale can be accurately found (see Fig. 113).

Verification by Quartz Plates. The simplest and easiest method of scale verification, as well as the most accurate, is by means of carefully standardized quartz plates. The cost of a sufficient number of plates to standardize the entire scale, however, is prohibitive, so that the chemist is usually content with a few standard plates for that portion of the scale most used, as 80 to 100 for cane sugar. The possession of a few carefully standardized quartz plates is a necessity for accurate saccharimetric work, not so much for standardization (since the constant error of the scale need be determined but once), but for the determination of 0 point, which is necessary with each set of observations.

The standard quartz plates furnished by instrument makers are mounted in metal tubes upon which is stamped the reading that the plates should give upon the particular saccharimeter scale. It is important that this reading be verified by some testing bureau, as slight errors in marking or faults in optical homogeneity of the plate are not uncommon. The surface of the plate when placed in the instrument must be perpendicular to the beams of polarized light which traverse it; for this reason the plates should never be loose in their mountings.

On the other hand, the mounting must not press too tightly upon the plate, as optical errors might be produced in the quartz. Rotation of the plate about the axis of its tube should cause no change in the field at the end point. The plate when being used should be brought as close to the analyzer diaphragm as possible in order to give the greatest spread to the cone of light rays emanating from each part of the field. Care must be taken that the standard plate during polarization have exactly the same temperature as that of the quartz wedges of the instrument. If the plate has a temperature above that of the wedges, it will give a reading higher than its true value. The temperature polarization coefficient of quartz is 0.000136, so that the polarization of a plate reading 100° V. at 20° C. would be, for 30° C.

$$100\ \{1 + (0.000136)\ (30° - 20°)\} = 100.14°\ \text{V.}$$

If plate and instrument are of different temperature, the plate should remain several hours in the trough of the saccharimeter before using, that sufficient time may be given for it to acquire the same temperature. Although it is necessary that quartz plate and wedge system have the same temperature, it is not essential that this be the standard temperature for the instrument, since the variations due to temperature are practically the same for plate as for wedge. The slight differences due to effect of temperature upon shape of quartz wedge and upon expansion of nickeline scale are expressed by the formula (Schönrock), $V_{20} = V_t + V_t\ 0.000005\ (t - 20)$, in which V_{20} and V_t are the readings of the plate at 20° C. and t° C., respectively. A standard plate polarizing 100° V. at 20° C. would accordingly polarize 99.99° V. at 40° C. (plates and wedges in each case at same temperature), a variation of 0.01° V. for 20° C. difference, which is negligible in practical work.

In the use of standard quartz plates it is important to know whether they have been calibrated according to the conversion factor of Schönrock (34.657°) or according to that of Bates and Jackson (34.620°). At the Eighth Session of the International Commission Schönrock recommended that the plates calibrated by the Bates-Jackson factor be marked " IP " near the edge, together with the serial number, the date, and the distinguishing mark of the standardizing bureau.

Verification by Pure Sucrose. A second means of verifying the saccharimeter scale is with chemically pure sucrose. The preparation of sucrose of requisite purity is a matter of some difficulty; the method of the International Commission for Unifying Methods of Sugar Analysis[39] is as follows:

[39] " Proceedings of Paris Meeting," July 24, 1900.

The purest commercial sugar is purified in the following manner: Prepare a hot saturated aqueous solution, precipitate the sugar with absolute ethyl alcohol, spin the sugar carefully in a small centrifugal machine, and wash in the latter with absolute alcohol. Redissolve the sugar obtained in water, again precipitate the saturated solution with alcohol, and wash as above. Dry the second crop of crystals between blotting paper, and preserve in glass vessels for use. Determine the moisture still contained in the sugar and take this into account when weighing the sugar which is to be used.

If a hand centrifugal is not available, the fine crystals of sugar may be filtered and washed free of sirup upon a Büchner funnel. In saturating the sugar solution before precipitation with alcohol, it is well not to heat above 80° C. The sugar solution thus prepared is filtered through a hot-water funnel into the alcohol, stirring vigorously. In this way the sugar is precipitated in the form of fine crystals which are easily dried in the air. Moisture is determined by drying at 50° C. under a maximum pressure of 40 mm. of mercury.[37] The low temperature is necessary because highly purified sucrose decomposes more readily at high temperature than ordinary refined sugar.[38]

The sugar may also be purified by recrystallization from water solution. Balch and Hill[39] used the following method. A 50 per cent solution of the sugar in distilled water is treated with a generous amount of vegetable decolorizing carbon and filtered through a compactly woven paper filter precoated with a small quantity of kieselguhr. This treatment removes mineral and colloidal matter. The solution is made slightly alkaline with ammonia to prevent inversion during evaporation. It is then refiltered through a thin pad of asbestos fiber directly into a distilling flask in which it is concentrated under a pressure of about 25 mm. of mercury at a temperature below 35° C. When the concentration reaches approximately 78 per cent solids, the sirup is transferred to a glass precipitating jar, seeded with recrystallized sucrose, and stirred at frequent intervals until the greater part of the crystallization is completed, which requires about 2 hours. The massecuite is allowed to stand several hours longer to complete crystallization, and is then purged in a centrifugal with a bronze basket lined with copper gauze over which is placed a layer of fine-mesh silk bolting cloth. The sugar is washed in the centrifuge with acid-free alcohol (70–95 per cent). It is then removed from the basket, treated with a sufficient quantity of 95 per cent alcohol to obtain a flowing mixture, again centrifuged, and washed with alcohol. The sucrose is dried on

[37] *J. Assoc. Official Agr. Chem.*, 12, 117 (1929).
[38] *U. S. Bur. Standards Sci. Paper* 268, 1917.
[39] *J. Assoc. Official Agr. Chem.*, 12, 108 (1929).

shallow enameled trays covered with filter paper, and passed through a 40-mesh phosphor-bronze screen. Before polarization it is further dried in a vacuum desiccator over phosphorus pentoxide. Sugar prepared in the manner just described showed, after two recrystallizations, a maximum of 0.0015 per cent ash, 0.002 per cent moisture, and 0.0015 per cent invert sugar, or a total of 0.005 per cent impurities, which is much less than the error of ordinary saccharimetric observation.

A more elaborate procedure of recrystallization from water is described by Bates and Jackson.[38] It leads to a product practically free from invert sugar and with a moisture content below 0.001 per cent.

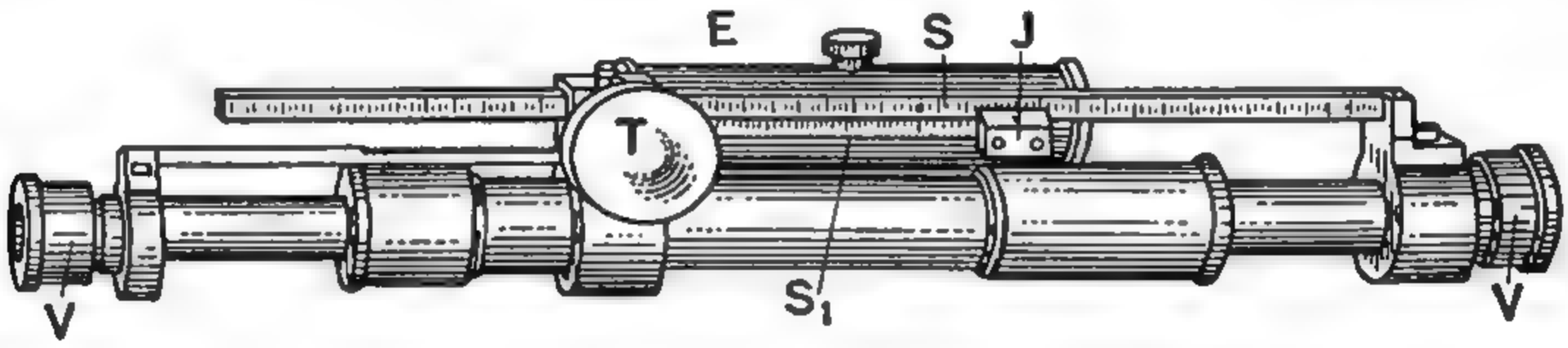
In the selection of sugar for purification, the finest grades of small domino sugar (polarizing 99.90 to 99.95) have been found to give the best results. Rock-candy crystals, which are sometimes recommended, should never be used; they frequently contain perceptible quantities of acid, with the result that inversion takes place during purification. Complete absence of acidity in sugar and alcohol is necessary.

To verify the 100° point of the saccharimeter scale, the normal weight of sugar is weighed into a 100-ml. flask, dissolved in distilled water, and the solution made up to volume, care being taken that the liquid is well mixed before making up the last few milliliters. The solution, which must be perfectly clear, is then polarized in a 200-mm. tube. The conditions of weight, volume, and temperature required for the saccharimeter must be rigidly observed; the flasks and tubes employed should have been previously calibrated. The average of ten readings is taken and this result corrected for the moisture in the sugar, the amount of which must be determined in a separate portion with each set of observations. The sugar used for polarization should not be dried in a heated-air or water bath owing to the danger of slight changes in composition. If the vernier of the scale is set at 0 when the field is matched, the polarization of the sugar corrected for moisture should be exactly 100. In the same manner, other divisions of the saccharimeter scale can be verified by taking fractions of the normal weight (e.g., normal weight $\times$ 0.85 = 85° point of scale, etc.; see Table XXXII).

Verification by Control Tube. The most convenient means of verifying the scale divisions of a saccharimeter when using sucrose is by means of the Schmidt and Haensch control tube.[40] This method presents the advantage that perfectly pure sucrose does not need to be used; in addition to this, but very few solutions are necessary for verifying the entire scale.

[40] *Z. Instrumentenk.*, 4, 169 (1884).

The control observation tube according to the latest form is shown in Fig. 112. It is telescopic in construction and can be adjusted so as to give a column of solution for any length between 210 and 420 mm. The length of solution, which is regulated by the screw *T*, is read off upon the scale *S* by means of the vernier *J* to 0.1 mm. The tube is provided with a reservoir *E*, which does not serve for filling, but simply receives the overflow of solution as the tube is shortened. To fill the tube, it is drawn out to its full length by means of screw *T*. Then the cover on reservoir *E* is unscrewed and the hole closed with a cork or with the finger. The tube is now filled like the ordinary polarimeter tube. The cork is removed and the tube shortened as much as possible, being held in such a way that the displaced sugar solution runs out of the reservoir without wetting the tube itself. Then the cover is replaced on *E* to prevent evaporation of the solution as far as possible.



(Courtesy of Akalos, Inc.)

FIG. 112. Control tube for verifying scales of saccharimeters.

In using the control tube, it is best to begin at the 100° point (which is supposed to have been previously verified) of the saccharimeter scale and work downwards. A sugar solution is first made up of such concentration as to give a reading of 100° at about 400-mm. length of tube. This will be sufficient to test the scale the few divisions above 100 and all divisions below 100 to 55. If the reading, for example, is 100 at 400 mm. upon the tube scale, it should read 105 at 420 mm., 95 at 380 mm., etc. If a deviation is found at any division from the calculated value, other readings should be made at neighboring points of the scale to determine the position of maximum error. After testing the scale to the fifty-fifth division (220 mm.), another solution must be prepared which will give a reading of 55 at about 400 mm. and the scale tested down to 30. By proceeding in this way, always making the final point of one series the starting point of the next, the scale can be tested its entire length with five solutions. Landolt[41] has given a table of concentration for solutions to be used with the control tube in testing the Ventzke scale (see p. 191).

The scale errors may be determined more conveniently, but less

[41] " Das optische Drehungsvermögen," 2nd ed., p. 341, 1898.

Number	Grams of Sucrose in 100 ml. of Solution	Starting Point for Verification, °V.	Range of Scale Divisions for Verification
1	12.53	100	95, 90, 85, .. 60, 55
2	6.89	55	50, 45, 40, 35, 30
3	3.76	30	25, 20, 16
4	2.00	16	15, 10, 9
5	1.13	9	5

accurately, by using the variable length between 210 and 0 mm. In this case only one solution is required, preferably one containing 26 g. sucrose in 100 ml. The inside of the tube is first carefully cleaned with a tube brush. The tube is shortened until the inner tube protrudes sufficiently so that the special holder and cover provided with the apparatus can be screwed on. The other end of the inner tube is left open, because no solution can enter into it. The tube is drawn out to its full length, filled with the sugar solution, and then used as described above for examining the entire scale of the saccharimeter. The length of the telescope tube, in millimeters, is found on another scale, opposite the 420 to 210 mm. scale.

In making the readings, the scale of the saccharimeter should first be set at the division which it is desired to verify and then the screw of the observation tube turned until the length of sugar solution gives a matched field. The reading upon the scale of the observation tube is then taken by means of a magnifying glass. The observed length of tube at any division in percentage of the observed length for the 100° point gives the actual value of the scale division. To distribute and equalize the errors due to changes in room temperature, warmth imparted to the tube by the hand in making the adjustment, eye fatigue, and other causes, it is well to proceed forward and backward along the tube and not make all the observations for one point at one time. It is desirable that several sets of readings be made upon different days and by different observers, the average of the several series being taken. The results obtained upon one of the saccharimeters belonging to the New York Sugar Trade Laboratory and shown in Table XXXIII will illustrate the method.

The results show great exactness of graduation, the error in no instance exceeding 0.05°.

By marking the degrees of the saccharimeter scale upon a straight line and laying off the observed errors above or below this line for their respective scale divisions, the curve connecting the error points will give the correction for any degree of the scale. The diagram of Fig. 113 for the observations of Table XXXIII illustrates the method.

TABLE XXXIII

Verification of S. & H. Saccharimeter, No. 7075

Series 1

Scale Division of Saccharimeter	Reading of Scale of Control Tube (average of 10 readings)	Value of Scale Division (in terms of 100° point)
	mm.	
100	396.365	100.000
95	376.495	94.987
90	356.740	90.003
85	336.930	85.005
80	316.975	79.972
75	297.120	74.962
70	277.290	69.957
65	257.465	64.957
60	237.710	59.972

Average of Series

Series	Scale Division of Saccharimeter								
	100	95	90	85	80	75	70	65	60
1	100	94.987	90.003	85.005	79.972	74.962	69.957	64.957	59.972
2		95.022	90.028	85.010	80.033	75.000	69.990	64.988	59.960
3		95.008	90.005	85.005	79.985	74.998	70.003	65.012	59.980
4		94.995	90.023	85.005	79.990	74.993	69.980	64.968	.....
5		94.985	90.015	84.985	79.985	75.003	69.995	64.997	59.990
6		95.037	90.025	85.038	80.038	75.028	70.008	64.990	60.002
Final average	100.000	95.002	90.017	85.007	80.001	74.997	69.989	64.985	59.981

A similar average made upon another S. & H. saccharimeter (No. 6920) gave

	100.000	95.004	90.034	85.041	80.050	75.028	70.035	65.031	60.015

To verify the scales of a double-wedge saccharimeter, the scales of both wedges are first set at 0 with their verniers for the matched field, any deviation of the 0 point being corrected by the regulating screw. The working-wedge scale is then verified and its curve of error determined by the control tube in the manner described. The control scale is then compared with the corrected readings of the working scale and its own error curve plotted. A still better direct method is to set the working wedge at 100 and then verify the control scale from the 0 division upwards by means of the control tube, using the same solutions as for verifying the working scale. If the tube, for example, with a length of 400 mm., gives a reading of 100° V. on the working-wedge scale with control-wedge scale at 0°, then with the working-

wedge scale at 100° V. the control-wedge scale should read 5 with a tube length of 380 mm., 10 with a length of 360 mm., etc.

The millimeter scale of the control tube should be verified before the instrument is put to use. The control tube can be employed only upon the large saccharimeters, which have a trough length of 420 mm.

Browne[42] has shown that the telescopic control tube is useful not only for verifying the scale of a single instrument by a single observer but also for comparing the scales of different saccharimeters by reading the control tube at each setting in several instruments in succession, or for determining the probable error for a given number of readings and the probable error in the readings of each observer, and

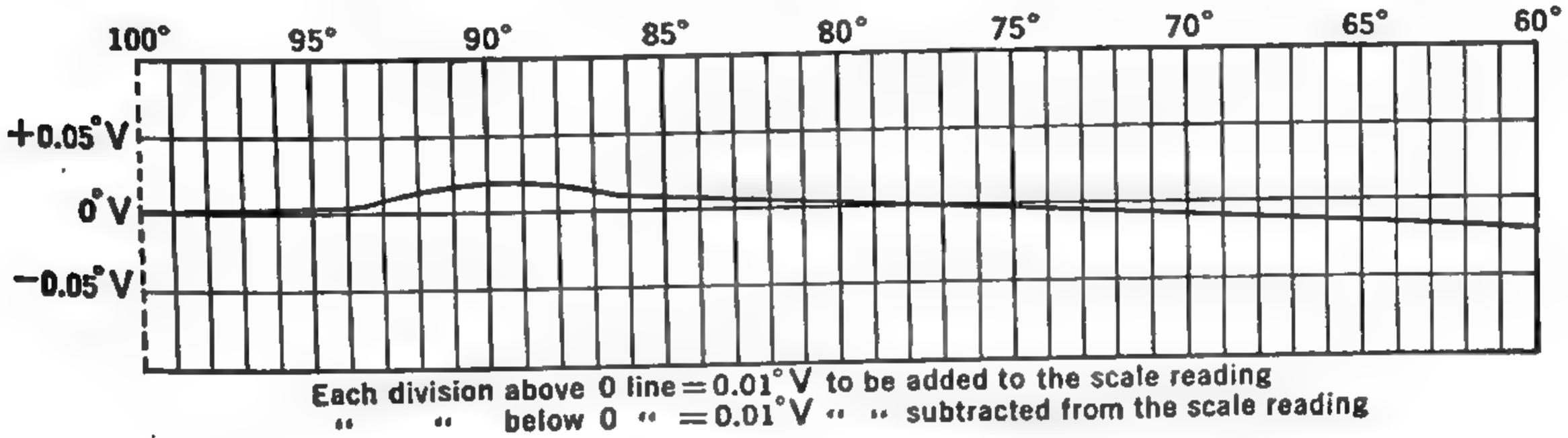


FIG. 113. Example of diagram for correcting saccharimeter readings.

finally for ascertaining the influence of peculiarities in the eyes of an observer. In this investigation it was noted that one observer obtained consistently lower values than another observer on one instrument, but consistently higher values on another instrument, the difference amounting in either case to about 0.04°, although the general averages for each observer on different instruments and for all observers on one instrument were in good agreement. This phenomenon was found to be due to the fact that in one instrument the half prism of the Lippich polarizer was on the left side and in the other instrument on the right side of the field. From this it must be concluded that, as long as the human eye is used for polarimetric observations, differences of several hundredths of a degree in the results of different observers can be expected even under the best conditions.

Verification by Scheibler's[43] Method of "Hundred Polarization." Another means of verifying the scale readings of a saccharimeter is Scheibler's so-called method of "hundred polarization." In this proc-

[42] *J. Ind. Eng. Chem.*, 12, 792 (1920).
[43] *Z. Zuckerfabr. deut. Reiches*, 21, 320 (1871).

ess of verification the polarization of the raw sugar or other product is first determined and then the calculated amount of substance weighed out which should give a polarization of exactly 100. Thus: if a normal weight of 26 g. of a sugar dissolved to 100 ml. polarizes 82.5, then $\frac{26 \times 100}{82.5} = 31.515$ g., the weight of sugar dissolved to 100 ml. necessary to polarize exactly 100. If the polarization obtained by the calculated weight of sugar is found to be 100, then the original scale reading of the saccharimeter is verified.

Effect of Temperature upon the Reading of Saccharimeter Scales

In the polarization of sugars and other materials upon quartz-wedge saccharimeters, the effect of temperature upon the scale reading is a most important factor. The saccharimeter is graduated to be used at a fixed temperature (17.5° C. or 20° C.), and in the most carefully regulated sugar laboratories this temperature is maintained throughout the year. But very few laboratories, however, are equipped with the necessary appliances for maintaining a temperature of 20° C. in summer, and the influence of temperature changes upon the saccharimetric readings and the methods for correcting the errors of the same should therefore be considered.

Temperature Coefficient of Quartz. The changes in specific rotation of sugars with variation in temperature are considered on p. 271. These changes apply to measurements made upon any kind of polariscope. But with the saccharimeter, as distinguished from the rotating polariscope, there must be considered an additional error due to the influence of temperature upon the quartz compensation of the instrument. This influence has been shown by Schönrock[44] to be threefold. There is (1) the change in shape of the wedge by expansion or contraction. The coefficient of expansion per 1° C. of quartz perpendicular to its axis (η) is 0.000013, and parallel to its axis (η') is 0.000007. The polarization value of the 100 point of the scale through change in shape of the wedge decreases with increasing temperature by $\eta' - \eta$, or by the coefficient —0.000006. There is (2) the change per millimeter thickness in the specific rotation of quartz itself, which for each degree increase in temperature increases by the coefficient 0.000136. The combined temperature coefficient of the wedge system is therefore 0.000130. There is (3) the change due to the expansion and contraction of the material constituting the scale. The error due

[44] *Z. Ver. deut. Zucker-Ind.*, **54**, 521 (1904).

to this change, together with that resulting from atmospheric humidity, was so great with the old ivory scales that the latter have been replaced in some saccharimeters with the alloy nickeline which has an expansion coefficient per 1° C. of 0.000018. The total correction, therefore, for a quartz-wedge saccharimeter with nickeline scale is 0.000148. The polarization value w for any temperature t is then expressed by the equation $w^t = w^{20} \{1 + 0.000148\ (t - 20)\}$. In the case of a glass scale, used in some saccharimeters, the coefficient becomes 0.000138 (expansion coefficient of glass = 0.000008). With saccharimeters whose scale is etched directly upon the wedge itself, as it is in many modern instruments, the coefficient remains 0.000130.

The above increase in polarization of quartz with increase in temperature necessarily produces a lowering in the readings of the saccharimeter scale, since a smaller thickness of quartz is required for compensation. With sugars which undergo a decrease in specific rotation with increase in temperature, the combined influences are in one direction and the error thus introduced may be considerable. With sucrose, for example, the temperature coefficient of polarization becomes at 10° C. 0.000390 (0.000148 + 0.000242), at 20° C. 0.000332 (0.000148 + 0.000184), and at 30° C. 0.000269 (0.000148 + 0.000121).

Temperature Coefficient of Sucrose. The variation in the Ventzke reading of the normal weight of pure sucrose for 1° C. change in temperature has been found by different authorities to be as follows:

Andrews[45]	0.0300
The United States Coast and Geodetic Survey	0.0293
Wiley[46]	0.0314
Prinsen Geerligs[47]	0.0300
Watts and Tempany[48]	0.0310
Average =	0.0303

The average temperature coefficient of the above is therefore 0.000303, which agrees with the figure of Schönrock for 25° C. (0.000148 + 0.000152) = 0.000300. For temperatures between 20° and 30° C. the general equation $V^{20} = V^t \{1 + 0.0003\ (t - 20)\}$ may be used for changing the Ventzke reading (V^t) of pure sucrose at any temperature t to the reading (V^{20}) at 20° C. The same formula may be used for degrees S. instead of V.

[45] *Technology Quarterly*, Mass. Inst. Technology, May, 1889, p. 367.
[46] *J. Am. Chem. Soc.*, 21, 568 (1899).
[47] *Arch. Suikerind.*, 11, II, 721 (1903).
[48] *West Indian Bull.*, 3, 140 (1906).

Temperature Coefficients of Other Sugars. The temperature coefficients of other common sugars for readings upon the Ventzke scale are given in Table XXXIV. The temperature coefficient for fructose and invert sugar are for readings made upon the negative scale of the saccharimeter. While the readings for these sugars decrease with rising temperature, the same as for the dextrorotatory sugars, the direction of the decrease in both cases is toward the 0 point and therefore in opposite sense to each other (as shown by the arrow points, → indicating a change from the left, and ← a change from the right toward the 0 point).

TABLE XXXIV

TEMPERATURE COEFFICIENTS OF DIFFERENT SUGARS FOR VENTZKE SCALE

Sugar	*A* $[\alpha]_D^{20°}$	*B* Change in $[\alpha]_D^{20°}$ for 1° C. Increase	*C* Temperature Coefficient $\frac{B}{A}$	Temperature Coefficient of Reading upon Ventzke Scale for 1° C. Increase. C + Coefficient for Quartz (−0.000148)	
Fructose.....	−92.88	+0.594	−0.006395	−0.006543	→0
Invert sugar..	−20.07	+0.297	−0.014798	−0.014946	→0
Lactose.....	+52.53	−0.070	−0.001332	−0.001480	0←
Maltose.....	+138.04	−0.095	−0.000688	−0.000836	0←
Glucose.....	+53.23	No change	No change	−0.000148	0←

The figures given in this table are approximate; the specific rotation as well as the temperature effect usually vary with both concentration and range of temperature. The coefficients given are applicable also to readings in degrees S.

If a mixture of sugars is polarized upon a saccharimeter, the combined influence of the temperature coefficients of each sugar must be considered. To arrive at a better understanding of the use of such coefficients the following special problem is considered:

It is desired to find the amount of fructose and of invert sugar which, mixed with 26 g. of pure sucrose, will give a constant saccharimeter reading over a considerable temperature range.

It has been shown that 26 g. of pure sucrose, reading 100° S. at 20° C., undergoes a decrease of 0.03° S. with 1° C. increase in temperature. Since a fructose solution reading −1° S. undergoes a decrease in polarization of 0.00654° S. (Table XXXIV), then $\frac{0.03}{-0.00654} = -4.59°$ S., the scale reading of the required amount of fructose. Since 0.1880 g. of fructose in 100 ml. reads −1° S. at 20° C. in a 200-mm. tube (formula of Jackson and Mathews), then $4.59 \times 0.1880 = 0.863$ g., the required amount of fructose. Therefore,

26 g. sucrose and 0.863 g. fructose (3.32 per cent of the weight of sucrose) will give a constant saccharimeter reading over a considerable temperature range.

In the same way for invert sugar, $\frac{0.03}{-0.01495} = -2.01°$ S., the scale reading of the required amount of invert sugar. Since 0.8625 g. invert sugar in 100 ml. reads −1° S. at 20° C. in a 200-mm. tube, then $2.01 \times 0.8625 = 1.734$ g., the required amount of invert sugar. Therefore, 26 g. sucrose and 1.738 g. invert sugar (6.67 per cent of the weight of sucrose) will give a constant saccharimeter reading over a considerable temperature range.

The amounts of fructose or of invert sugar which, when added to sucrose, give a mixture of constant rotation are only approximate, for the reasons stated above, and also because the specific rotation of sugar mixtures is not a strictly additive property.

The effect of 1° C. increase in temperature upon the reading of 1 per cent each of sucrose, fructose, and invert sugar for a normal weight of 26 g. in 100 ml. is given in Table XXXV.

TABLE XXXV

INFLUENCE OF TEMPERATURE UPON READING OF 1 PER CENT SUCROSE, FRUCTOSE, AND INVERT SUGAR FOR A NORMAL WEIGHT OF 26 G. SOLUTIONS MADE UP TO VOLUME AT TEMPERATURE OF POLARIZATION

$$\text{1 per cent sucrose} = \frac{0.03}{100} = -0.0003° \text{ S. for } 1° \text{ C. increase.}$$

$$\text{1 per cent fructose} = \frac{0.03}{3.32} = +0.0090° \text{ S. for } 1° \text{ C. increase.}$$

$$\text{1 per cent invert sugar} = \frac{0.03}{6.67} = +0.0045° \text{ S. for } 1° \text{ C. increase.}$$

(— denotes change toward the left; + denotes change toward the right.)

Since the influence of temperature upon the rotation of glucose is so small as to be negligible, the change in rotation for 1 per cent invert sugar, should be approximately the same as that for 0.5 per cent fructose, or +0.0045. This is actually found to be the case. The value 0.0045 is used in the formula given on p. 396 for correcting the direct polarization of raw sugars for the effect of temperature.

SHALL SACCHARIMETERS BE ADJUSTED TO VARIABLE TEMPERATURES?

The International Commission[49] has provided that "for laboratories in which temperatures are usually higher than 20° C., it is permissible to graduate saccharimeters at any suitable temperature, providing that the volume be completed and the polarization made at the

[49] "Proceedings of Paris Meeting," July 24, 1900.

same temperature." It was not stated at the time how this graduation is to be made. But at the Ninth Session of the Commission, in 1936,[50] the following resolution was adopted: "For saccharimeters used in tropical countries at $t°$ C., the fundamental values, 26.000 g., 100 metric cc., and 2.000 dm., shall be valid at $t°$ C. The polarization of the solution of pure sugar shall then be 100° S. Corresponding to this definition, the sugar value (of the quartz control plate) is $S_t = S_{20} + aS_{20}\ (t - 20)$. The magnitude of S_{20} is the sugar value of the same plate at 20° C. The constant a shall be fixed by the four National Physical Laboratories." The tentative value of a, found by Einsporn and Schönrock[51] at the Physikalisch-Technische Reichsanstalt, is 0.000296, valid between 17° and 33° C.

In other words, no change is to be made in the instrument itself, but a correction is applied to the reading, based on the change in the rotation of pure sucrose with change in temperature. (See pp. 390–392.) In routine factory work it is usually preferable to apply corrections automatically, and this could be done in the present case by altering the conditions of polarization, for example, by increasing the normal weight of sugar, or increasing the length of the observation tube, or decreasing the volume of the flask, any one of which means will bring the polarization of pure sucrose to 100 for any desired temperature above the standard. Since odd lengths of tube or volume of flask are undesirable as well as confusing, a change in the normal weight of sucrose is the simplest of all means of correction. The method of calculation can be understood from the following example:

What would be the normal weight at 25° C. for a quartz-wedge saccharimeter standardized at 20° C., the sucrose to be dissolved to 100 ml. in a flask calibrated at 20° C., and the solution to be polarized in a tube of 200-mm. length at 20° C.?

The temperature coefficient of the specific rotation of sucrose at 22.5° C. (midway between 20° and 25° C.) is −0.000168 (Schönrock). The temperature coefficient of the quartz wedge with nickeline scale is 0.000148. The expansion coefficient for the glass observation tube is 0.000008, and that for the volume of the solution in the flask 0.000024. The normal weight at 25° C. would then be

$$26.000\{1 + [(0.000148 + 0.000168 - 0.000008 + 0.000024)(25 - 20)]\}$$

or 26.043 g. A similar calculation for 30° C. gives a normal weight of 26.082 g.

When saccharimeters are employed constantly in the investigation

[50] *Intern. Sugar J.*, **39**, 28s (1937).
[51] *Z. Ver. deut. Zucker-Ind.*, **89**, 1 (1939).

of pure sucrose solutions, it might be advisable to make a change such as the above in the normal weight. But for varied work with different classes and mixtures of sugars whose specific rotations are affected in opposite ways by changes in temperature, it is inaccurate to make alterations based upon the change in properties of one single sugar. The results obtained upon saccharimeters differently standardized are then no longer comparable. The sucrose normal weight is frequently employed upon mixtures of sucrose with other sugars; in such cases changes in normal weight to correct for rotatory changes in the sucrose alone are wholly unwarranted. In view of the fact that the work of saccharimeters is usually of a varied nature, it seems best to leave the scale and standard conditions of the instrument unchanged. The chemist should work wherever possible under the conditions of temperature prescribed for his saccharimeter, and when this cannot be done he should correct his readings as well as possible by a factor established for the particular product which is being examined.

It must always be borne in mind that while the saccharimeter scale is established for the rotation of sucrose, its divisions indicate percentages only when pure sucrose is being polarized; in all other cases the scale division becomes a mere conventional number (degrees Ventzke, degrees polarization, degrees sugar scale, etc.) which the analyst must interpret according to his particular needs.

American Chemical Society Specifications for Saccharimeters

In 1920 a committee of the American Chemical Society prepared specifications[52] for the construction of saccharimeters. These specifications, as quoted below, were compiled from the opinions of many sugar chemists in the United States, Hawaii, Cuba, and other countries and are based upon the results of wide practical experience.

GENERAL CONSTRUCTION

The general construction of the saccharimeter outlined in these specifications should be as simple and substantial as possible. All parts of the instrument should be easily accessible and the number of bolts and screws for holding the parts in place should be reduced to the necessary minimum.

So far as possible the instrument should have smooth, plain surfaces and be without unnecessary ornamentation. An irregular ornamented surface affords grooves and crevices for the accumulation of dirt and is not easily cleaned.

The instrument should meet the requirements of exposure to a humid tropical climate and must be constructed to withstand corrosion.

[52] *J. Ind. Eng. Chem.*, 12, 440 (1920).

The construction of the saccharimeter, so far as possible, should be of such a type that repair parts can be furnished separately, thus obviating the expense, the delay, and the danger of shipping the entire instrument. Wherever this is not practicable manufacturers should undertake to make repairs in a satisfactory manner without undue delay.

HEIGHT

The standard height of most saccharimeters from table to center of field eyepiece is between 32 and 34 cm. This height is convenient for manipulation with the elbow of the operator resting upon the table and has found most general approval.

MOUNTING

The saccharimeter should be mounted upon a rigid trestle support and not upon a tripod. Instruments mounted upon tripods are unstable and easily turned out of alignment, the result being an error in the 0 point.

The base of the trestle should be a solid piece of metal at least 2 cm. thick, the bottom edge of which can rest at all points upon the table. A base elevated above the table by supporting knobs or projections lacks rigidity and has the disadvantage of permitting cover glasses and other objects to escape underneath.

As many chemists prefer to fasten their instruments to the table, the base of the trestle should be provided with slots or screw holes to facilitate this.

LAMP SUPPORT

The lamp end of the trestle should be designed to accommodate a strong removable bracket for the convenience of those who may wish to use it as a lamp support, thereby keeping lamp and instrument always in undisturbed alignment.

The holder of the lamp must be placed at the proper focal distance and should be adjustable. Bracket and holder should be designed so as to prevent transmission of heat to the polarizer of the instrument.

For rooms of constant temperature the lamp should be in a separate room.

TROUGH

The trough, or tube holder, should be of solid metal, in one piece, and sufficiently thick to prevent denting or bending under ordinary conditions of usage. The diameter of the trough at the top should be about 3 cm. and should be adjusted exactly to fit the end pieces of the observation tubes. The cross-section of the trough should be semicircular in shape. A wedge-shaped trough does not give the necessary support to inversion tubes or other tubes of unstable equilibrium.

The length of trough should be 42 cm. This length is necessary for the accommodation of the control tube which is used for verifying the accuracy of the scale. The short 20-cm. trough does not permit the use of the control

tube and is also inadequate for the polarization of sweet waters and other dilute solutions.

The base of the trough should be supported to the frame of the trestle and there should be a 2-mm. space between its ends and the rest of the instrument. This clearance allows the escape of any liquid which may be spilled in the trough and protects against warping of the trough and transmission of heat to the optical parts when polarizations are made at high temperature. The base of the trough must be parallel with the optical axis of the instrument.

The troughs should be made removable for the accommodation of other forms of tube supports or baths that may be needed in special cases. Owing to the corrosive action of solutions, which may be spilled inside of the trough, the screws for fastening the trough should be on the outside.

TROUGH COVER

The trough should be provided with a hinged cover for excluding light. The cover should be long enough to cover the 2-mm. space between trough and splash-glass holders, and should fold back to a horizontal position where it can be used in case of need as a receptacle for tubes.

The hinges of the cover should not be riveted. Many chemists find the trough cover an encumbrance, and for the convenience of such it should be easily removable.

For the convenience of those who use continuous, or side-filled, polarization tubes, a slotted cover should be provided as an optional accessory.

SPLASH GLASSES

The splash glasses at the ends of the trough, for protecting prisms and wedges against dust and drops of liquid, should be mounted in holders which can be quickly removed, cleaned, and replaced without the use of tools. Slip holders with a tension spring are most generally preferred, and they should be designed to prevent sticking.

The two holders should be as near alike as possible, at least 1.5 cm. deep and so constructed that glasses can easily be removed for cleaning. For ease of replacement when damaged, the splash glasses should be of the same size as the standard polariscope tube cover-glass.

QUARTZ WEDGES

For a commercial saccharimeter all chemists prefer the compensating single-wedge system. The wedge should be of sufficient length to give a range of scale from −35 to 115 sugar degrees.

If quartz of sufficient optical purity to give this lower range cannot be secured, a dextro quartz plate should be provided as an accessory for use in invert polarization.

The driving mechanism of the wedge should consist of a vertical rod supported to the front of the trestle frame and provided at the bottom with a

milled head about 7 cm. from the table and convenient for operation with either hand.

The spiral rack and pinion with which the driving rod connects should operate smoothly and without lost motion.

SCALE

The scale should be etched upon ground glass and read by transmitted light obtained from the light source of the instrument. The design of the instrument should be such that the scale can be illuminated, when continuous or control tubes are in upright position in the trough. The range of scale should be from −35 to 115. This upper limit is necessary for those who wish to determine purities without diluting below 20° Brix.

The scale should have an adjustable double vernier, for plus and minus degrees, and should easily be read to 0.05° to which end the magnifying power of the reading microscope should be amply large. The error of scale graduation should nowhere exceed 0.05°.

The adjusting screw for moving the vernier to the 0 point of the scale should operate positively in either direction. In some instruments a spring is designed to act when the adjusting screw is withdrawn. The objection to such a spring is its liability to stick and not to operate as intended.

Before shipment, the scale of each instrument should be carefully standardized at suitable intervals throughout its entire range and the standardization values should be incorporated permanently in some way upon a plate attached to the instrument.

PROTECTION CASE

Scale and wedges should be enclosed in a tight protection case to prevent deposition of dust or spattering with drops of liquid. The covering of the case should be easy to take off, when it is desired to gain access to scale or wedge, by the removal of a few fairly large-size screws. The rim of the protection case should have a covered aperture for inserting the key of the adjusting screw.

Whenever desired the front of the case should be provided with a small thermometer having a range of 10° to 40° C. and with its bulb near the quartz wedge. The thermometer should be arranged so that it can be read in a darkened compartment by light obtained from the lamp which illuminates the instrument.

SCREEN

The protection case should be designed to accommodate a removable screen to protect the eye which is not in use from the glare of the lamp. The screen should have a diameter of about 15 cm. at the level of the two oculars.

ANALYZER

While the analyzer is one of the parts which should require least attention, there are occasions when it needs to be adjusted. It should be made

fairly accessible and be provided with simple means for firmly securing the adjustment.

LIGHT FILTER

The light filter should be placed between the polarizer and light source of the instrument and should be so supported that it can be quickly thrown into the field or out without disturbing the position of the instrument.

The standard bichromate cell should consist of a glass tube 3 cm. long encased in a metal jacket with threaded ends to accommodate the screw caps for holding the glasses. The cell should have a sufficient diameter so as not to require refilling because of air bubbles during an ordinary campaign (or more than twice a year).

Many chemists desire a lightly ground glass over the aperture at the lamp end of the instrument to equalize the light. Such a glass, if properly tinted, might serve the double purpose of light filter and equalizer. As a matter of convenience the instrument should be equipped with a light filter consisting of a glass plate of the same depth of color and absorptive power as the standard light filter.

OCULARS

The oculars in front of the instrument for reading field and scale should focus with a screw motion. The sliding eyepiece is objectionable, owing to the ease with which it is pushed out of adjustment by the face of the observer.

The distance from center of field eyepiece to center of scale eyepiece varies in present instruments from about 3 to 6 cm. For convenience and rapidity in reading, the interval between the two eyepieces should lie within these limits.

FIELD

American chemists with few exceptions prefer the customary double field with vertical semicircular halves. The field should be of good size, sharply defined, and not obscured with the rim or halo of extraneous light, which results from improper optical construction.

POLARIZER

The preferences as to polarizer are divided between the Lippich and Jellett-Cornu prisms. Many chemists, while admitting certain advantages of the Lippich polarizer, complain of its frequent disintegration along the sharp edge of the half-prism upon which the telescope is focused, the result being an imperfect or shattered field. The disruption of the half-prism may result from a jar of the instrument or it may take place from no apparent cause. More saccharimeters are made unserviceable for this reason than for any other. The difficulty of repairing the damage, owing to the extreme fragility of the parts, renders the Lippich polarizer less suited for localities which are far distant from repair shops. Many chemists, on the other hand, who admit the greater stability of a modified Jellett-Cornu prism, complain of its lower

degree of sensitiveness owing to the pronounced dividing line of the field, the result either of too thick a film of balsam between the halves of the upper part of the prism or of imperfect alignment of the polarizer. The defects peculiar to each type of prism can largely be overcome by careful manufacture. There are many stable Lippich polarizers and many Jellett-Cornu prisms that are satisfactory in sensibility.

If manufacturers will make repairs rapidly and will furnish extra interchangeable half-prisms of easy adjustment, the usefulness of the Lippich polarizer will be much widened and the majority of chemists in fairly accessible localities will probably then prefer it. For remote tropical countries where repairs are difficult and time-consuming a modification of the Jellett-Cornu prism would probably be the better type. For these reasons the type of polarizer should in great measure be left optional with the purchaser. In their manufacture of polarizing prisms manufacturers should take every precaution to insure stability and to prevent drying out and cracking of the films of balsam cement.

A very serious complaint from tropical countries is the infection of the polarizer, analyzer, and other optical parts of the instrument by molds, the mycelia of which grow over the prisms, corroding their surface and obscuring the field. Efforts to prevent infection by enclosing the parts more adequately have not proved successful. The best means of preventing the growth of molds seems to be a construction that permits of easy accessibility and removal of parts for cleaning and for placing in desiccators during periods when the saccharimeter is not used.

MOUNTING OF PRISMS — Wax, as a mounting material for prisms, has proved objectionable in warm climates on account of its softening. A mounting in cork and plaster is said to be the most satisfactory.

HALF-SHADOW ANGLE — The fixed half-shadow angle of the polarizer in most saccharimeters varies from 5 to 9 angular degrees, the choice of angle by different manufacturers seeming to depend somewhat upon the length and pitch of the quartz wedge. It is probable that for general commercial purposes the half-shadow angle should fall within this range. The sensibility is greater but the intensity at the end point is less with the smaller half-shadow angle. Recent improvements in electric stereopticon lamps with concentrated filament and high candle power make it possible for manufacturers to adapt saccharimeters to a lower half-shadow angle than was formerly the case. For a normal weight of 26 g. the fixed half-shadow angle should have a magnitude of at least 7° for the average class of sugar factory raw products. The angle may be smaller than this for colorless products. The angle may also be reduced for raw products with instruments which are adapted to a normal weight of 20 or 16.29 g.

Chemists who work constantly with dark-colored sirups and molasses prefer a polarizer with a rather wide half-shadow angle. It would, therefore, be a distinct advantage if manufacturers could supply interchangeable polarizing prisms — one with a medium half-shadow angle between 5 and 8 angular degrees and another with a higher half-shadow angle between 9 and 12 degrees.

Polarizing prisms should be mounted in metal holders which can be easily removed and inserted and the adjustment of which can be quickly and securely fixed.

The sleeve, or cover, which protects the polarizer should be easy to take off by the removal of a few fairly large-size screws.

The standard temperature for the calibration of saccharimeters shall be 20° C. For laboratories working at a temperature materially different from this, correction of polarizations to 20° C. may be made at discretion by any of the following methods:

1 — By the use of a table of temperature corrections for each particular product.

2 — By changing the normal weight.

3 — By changing the capacity of normal flask.

4 — By changing length of normal tubes.

5 — By having a scale calibrated by the manufacturer so that it is correct for the temperature desired.

With the exception of the first method these methods of correction are strictly applicable only to products which contain no other optically active constituent than sucrose. For general sugar house and food products containing several sugars, in case constant temperature polarization at 20° C. is not permissible, Method 1 gives results which are nearest to those obtained at the standard temperature.

DESCRIPTION OF SACCHARIMETERS

Tint Saccharimeters

The saccharimeter of Soleil as modified by Ventzke and Scheibler in Germany and by Duboscq in France consists of an adaptation of the quartz-wedge compensation to the polariscope of Robiquet (p. 146).

The Soleil-Ventzke-Scheibler Saccharimeter. The construction and arrangement of the optical parts in the Soleil saccharimeter as modified by Ventzke and Scheibler are shown in Fig. 114. *A* is a Nicol prism and *B* a plate of left or right rotating quartz cut perpendicular to its optical axis; these constitute the tint producer and are mounted in a movable sleeve which can be rotated by a rod and pinion from *J*. *C* is a condensing lens, *D* the polarizer, and *E* a Soleil double quartz plate (p. 147). The quartz compensation is at *F*, the analyzer at *G*, and telescope at *H*. In using the instrument the telescope is focused upon the bi-quartz plate, so that the dividing line is sharply defined. The 0 point of the scale is then determined by turning *K* until both sides of the field have the same tint (in the manner described on p. 148). By rotating the regulator or tint producer from *J*, the tint which is

most sensitive to the eye of the observer is obtained. This tint, which is different for different eyes, is usually of a very delicate violet or pearl color; it will vary, of course, according to the angle with which the Nicol *A* is set with reference to the Nicol *D* of the polarizer. In

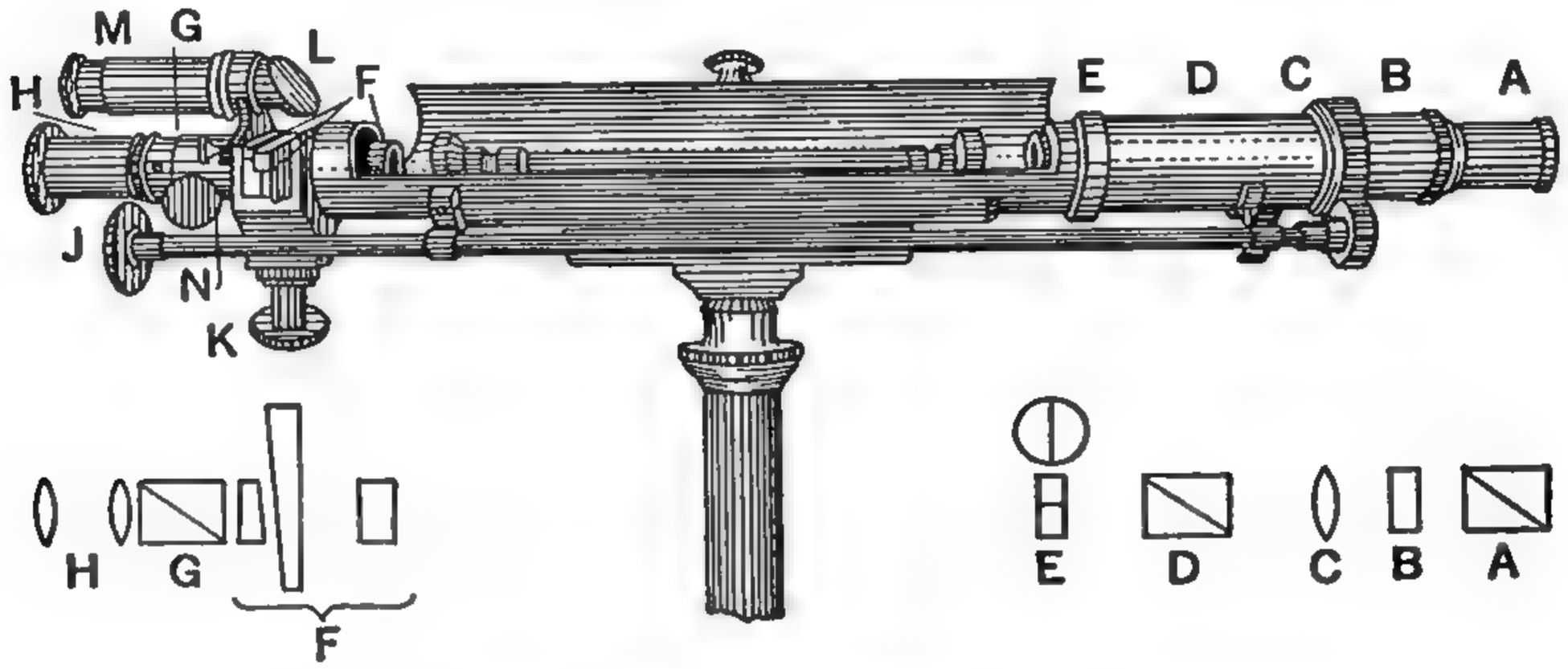


FIG. 114. Soleil-Ventzke-Scheibler tint saccharimeter.

order to remove the disturbances in transition tint due to colored solutions (which cannot be remedied in the Robiquet polariscope), the adjustment of the regulator is changed until the tint is again of greatest sensitiveness. With very dark solutions the transition tint is almost a shadow owing to the absorption of color.

The Soleil-Duboscq Saccharimeter. The Soleil saccharimeter as modified by Duboscq, the type of tint instrument used in France, differs from the form previously described in that the Nicol producing

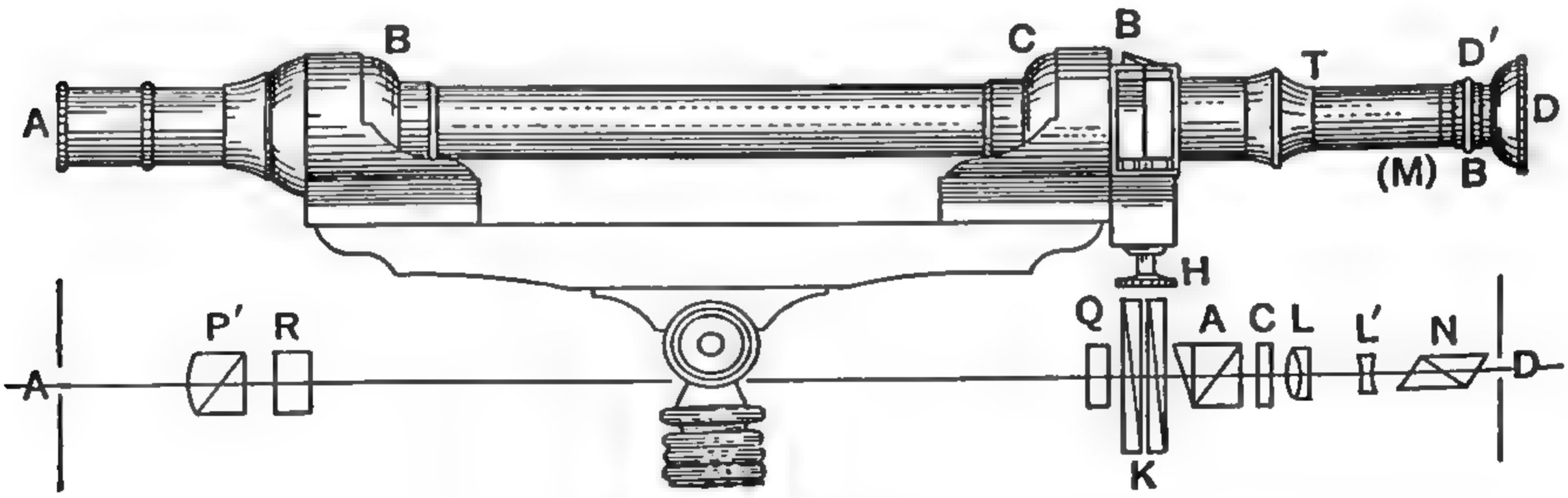


FIG. 115. Soleil-Duboscq tint saccharimeter

the sensitive tint is situated in the eyepiece of the telescope, as shown by *N* in Fig. 115. The latter is rotated by a milled ring *B* until the sensitive tint is produced with the quartz plate *C*, which in the Duboscq

instrument is situated between the analyzer and the objective of the telescope. The telescope is focused upon the Soleil double plate at *R* by moving the eyepiece *D* in or out; longitudinal guides prevent any lateral rotation which might disturb the tint. In the Duboscq instrument the two wedges of the compensator are of equal size and, being driven past each other by the pinion in opposite directions, give a stratum of quartz of variable thickness. A scale and vernier, which follow the wedges in their movement, indicate the reading.

According to Landolt[53] the average error of adjustment with the Soleil saccharimeter is $\pm 0.2°$ of the scale. The instrument has the same objection as the Robiquet polarimeter, in being unsuited to the color-blind. The adjustment of end point to color is also much more fatiguing to the eye than adjustment to uniformity of shade. Owing to these objections the color saccharimeter, which up to about 1890 was the standard instrument, has been entirely supplanted by the half-shadow type of apparatus. In fact, its use has been condemned by the Testing Bureau of the German Reich.

Half-Shadow Saccharimeters

The various types of half-shadow saccharimeter used at the present time consist simply of an adaptation of the quartz-wedge compensation to some one of the half-shadow polariscopes previously described. The principal forms are the double-field saccharimeter with Jellett-Cornu polarizer; the double-, triple-, and concentric-field saccharimeters with Laurent plate; and the double- and triple-field instruments with Lippich polarizer.

Saccharimeter with Jellett-Cornu Prism. A single-wedge half-shadow saccharimeter with Jellett-Cornu prism as polarizer is shown in Fig. 116.

The above saccharimeter, which around the close of the last century was the standard form of instrument employing the Ventzke scale, has been largely replaced by German and Czechoslovakian manufacturers with saccharimeters using the Lippich polarizer. Some instrument makers in other countries still use the Cornu polarizer or a modification of it.

Laurent's Saccharimeter. As a type of the saccharimeters constructed by French instrument makers, the Laurent instrument shown in Fig. 117 is described. The arrangement of polarizer, half-wave plate, and device for regulating the half-shadow angle is identical with that

[53] "Das optische Drehungsvermögen," 2nd ed., p. 348, 1898.

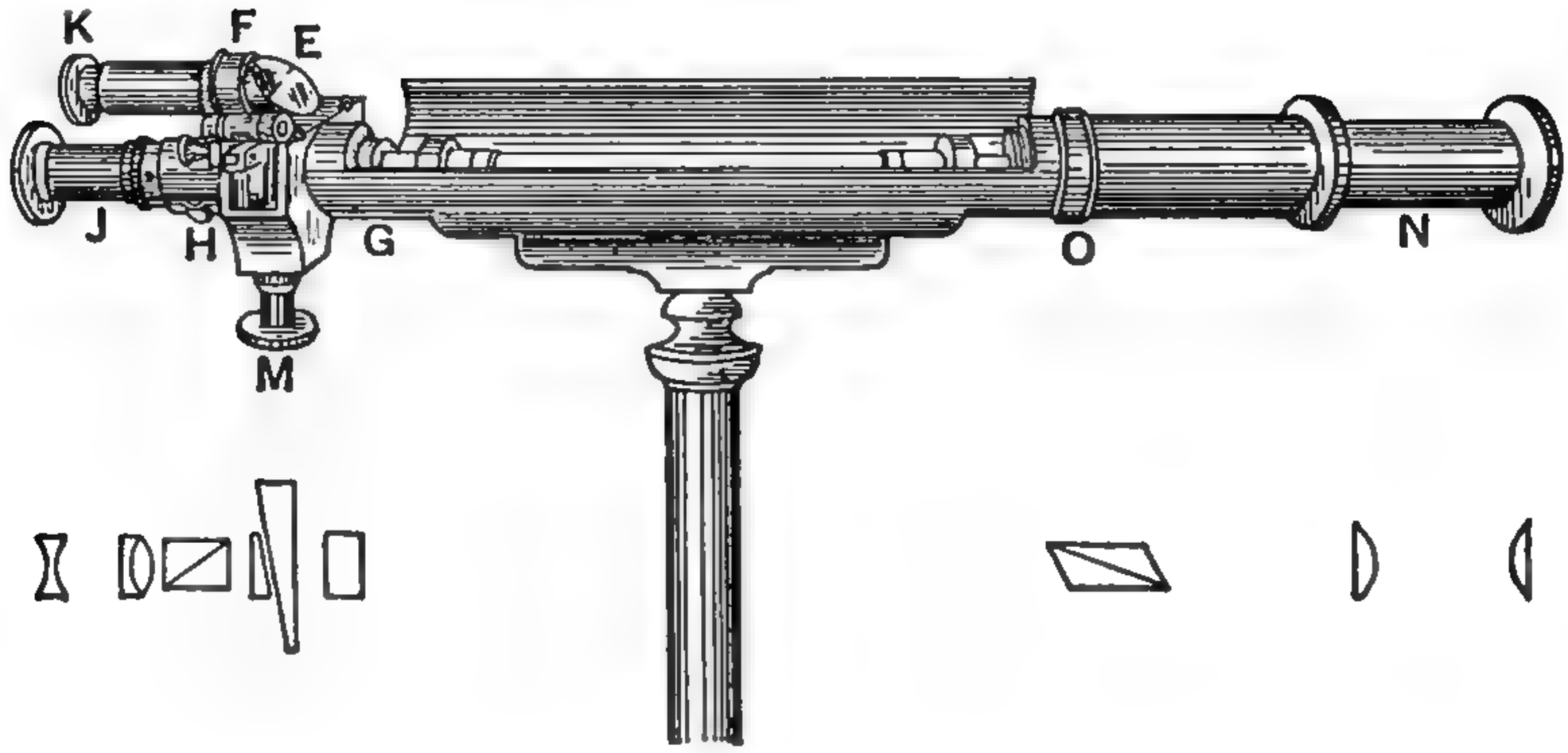


FIG. 116. Single-wedge saccharimeter with Jellett-Cornu prism.

N, sliding sleeve containing condensing lens; *O*, modified Jellett-Cornu prism (Schmidt & Haensch prism); *E*, *F*, parts of quartz-wedge compensation; *H*, Analyzer; *J*, telescope, which is focused upon the dividing line of the split prism at *O*. *K*, microscope for reading scale.

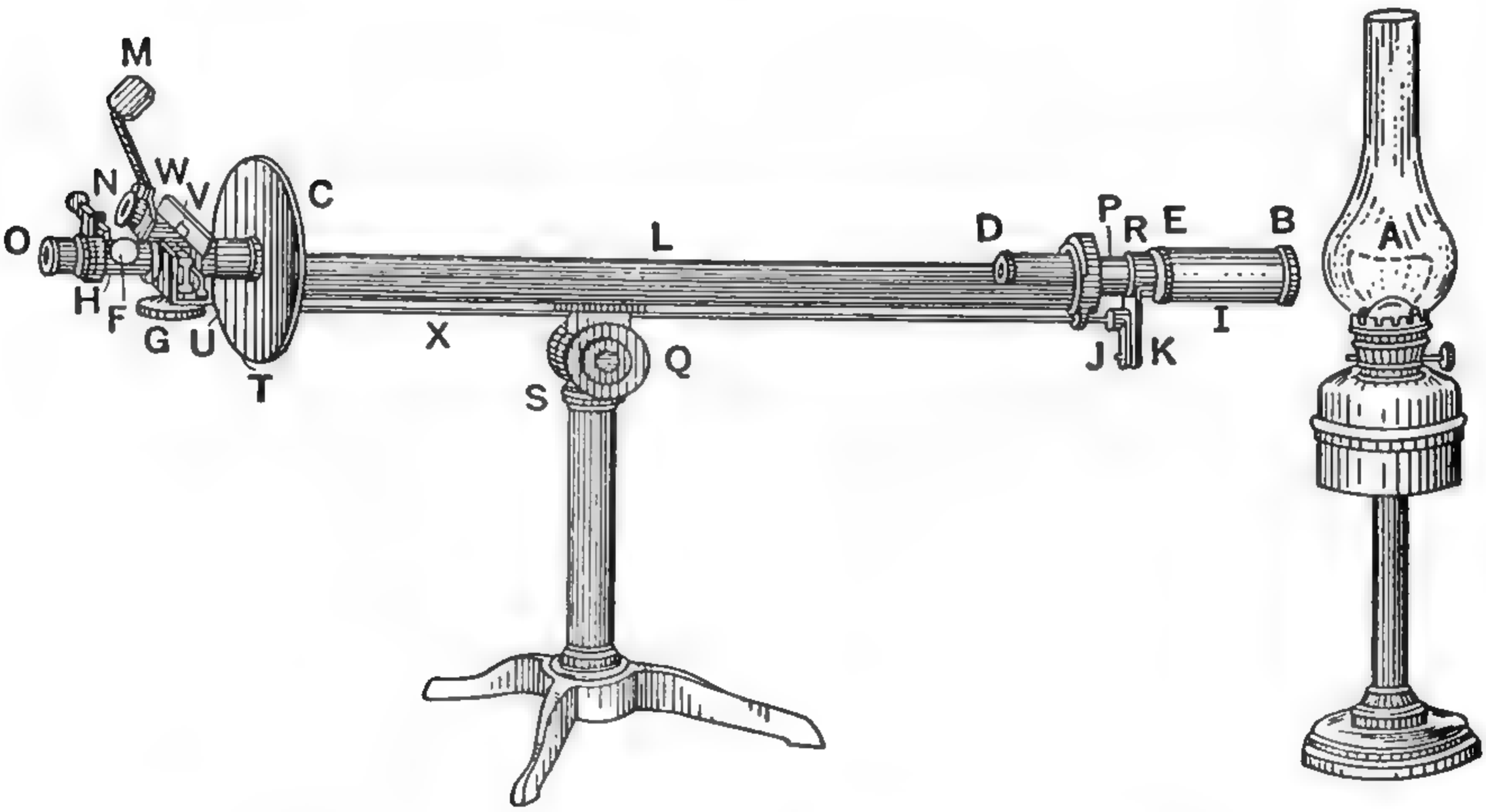


FIG. 117. Laurent's single-wedge saccharimeter.

A, lamp for producing white light (oil, gas, electricity, etc.), placed 200 mm. from *B*; *B*, *E* *R*, *K*, *J*, *X*, *U*, *D*, *L*, the same as under Laurent polarimeter (Fig. 101); *W*, saccharimeter scale, which with vernier *V* is illuminated by light reflected from *A* by the mirror *M*; *N*, magnifying glass for reading scale and vernier; *G*, screw for moving quartz wedges of the Soleil compensator.

of the Laurent polarimeter (Fig. 101). The divided circle and rotating analyzer of the latter, however, are replaced in the saccharimeter by the quartz-wedge compensation.

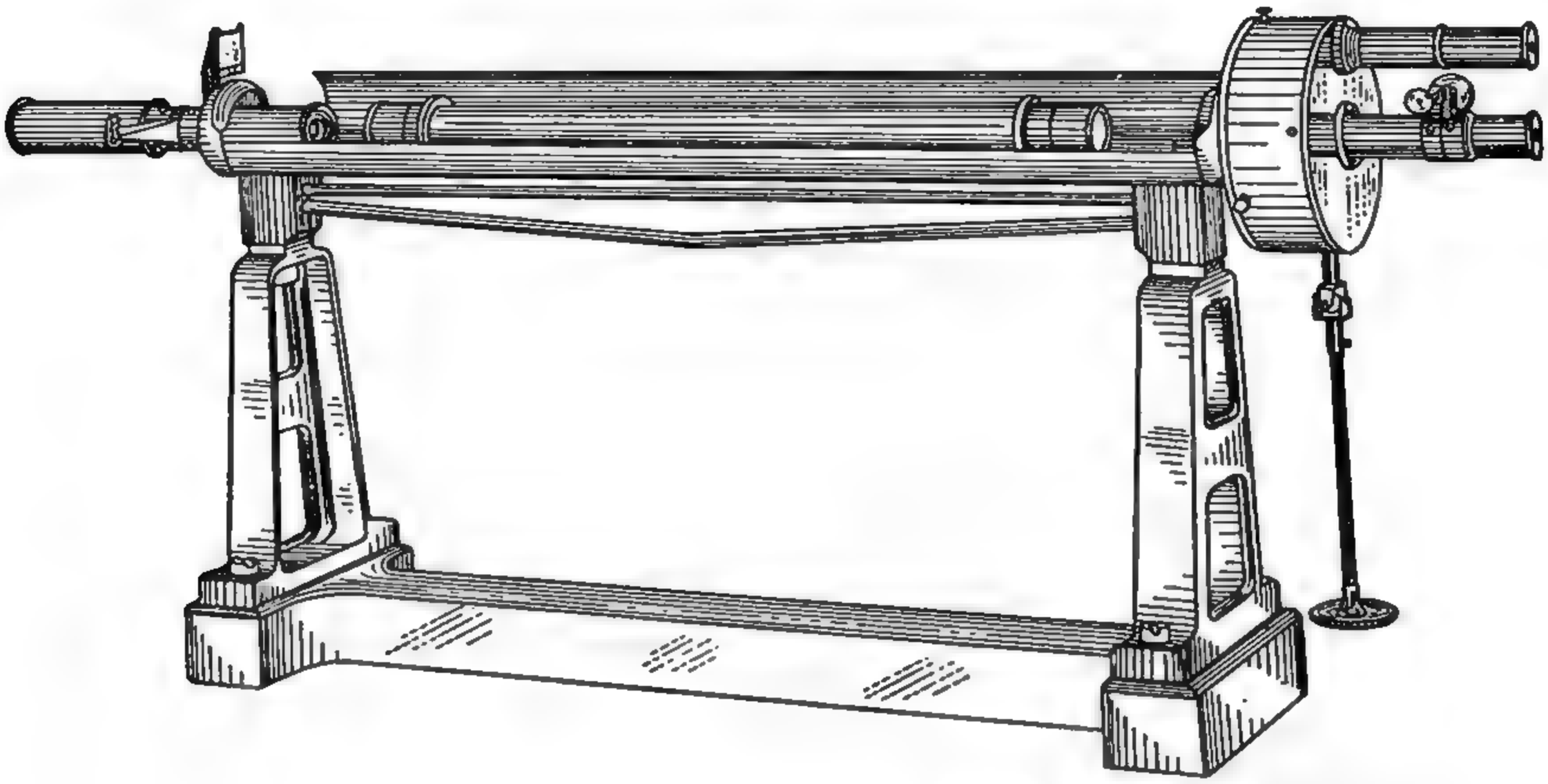
The saccharimeter is adjusted to its 0 point by first turning *G* until the two halves of the field agree in shade. If it should be found that one side of the field has more of a reddish tinge than the other at the end point, the screw *F*, which controls the analyzer, is turned so as to darken slightly the side of the field most colored. The screw *G* is then turned again to equality of shade; if there is still a difference in color, *F* is moved slightly as before, and *G* again turned to equality of shade. By proceeding cautiously in this way the observer will at length reach the point where both sides of the field correspond in shade and color. When this point is reached the screw *T* is turned until the 0 of the scale coincides with the 0 of the vernier. This adjustment should be verified by taking a number of check readings.

The 100° point of the Laurent saccharimeter scale corresponds to a rotation of 21° 40′, the value originally given by French physicists to the rotation of the 1-mm. plate of quartz. The normal weight for this angular displacement, as previously noted, is 16.269 g. sucrose for 100 ml. polarized in the 200-mm. tube. The Laurent saccharimeter is also manufactured with scales adapted to the normal weight of 20 g. or 10 g. The instrument is provided with double or triple field, as desired. The scale divisions extend from 0 to 110 to the right.

"*Plaque Type.*" The 100° point of the Laurent saccharimeter is verified by a standard plate of quartz reading 21° 40′, circular scale, for sodium light. This standard plate "plaque type" also serves for the polarization of levorotatory solutions. With the plate in the trough of the instrument, the 0 point of the scale is transferred to 100; levorotatory solutions are then simply read backwards upon the scale, the reading being the difference between readings of plate and solution. A solution, for example, reading 67.4 with the 100° plate in position has a polarization of —32.6. This method of polarizing levorotatory solutions is applicable, of course, to all single-wedge saccharimeters.

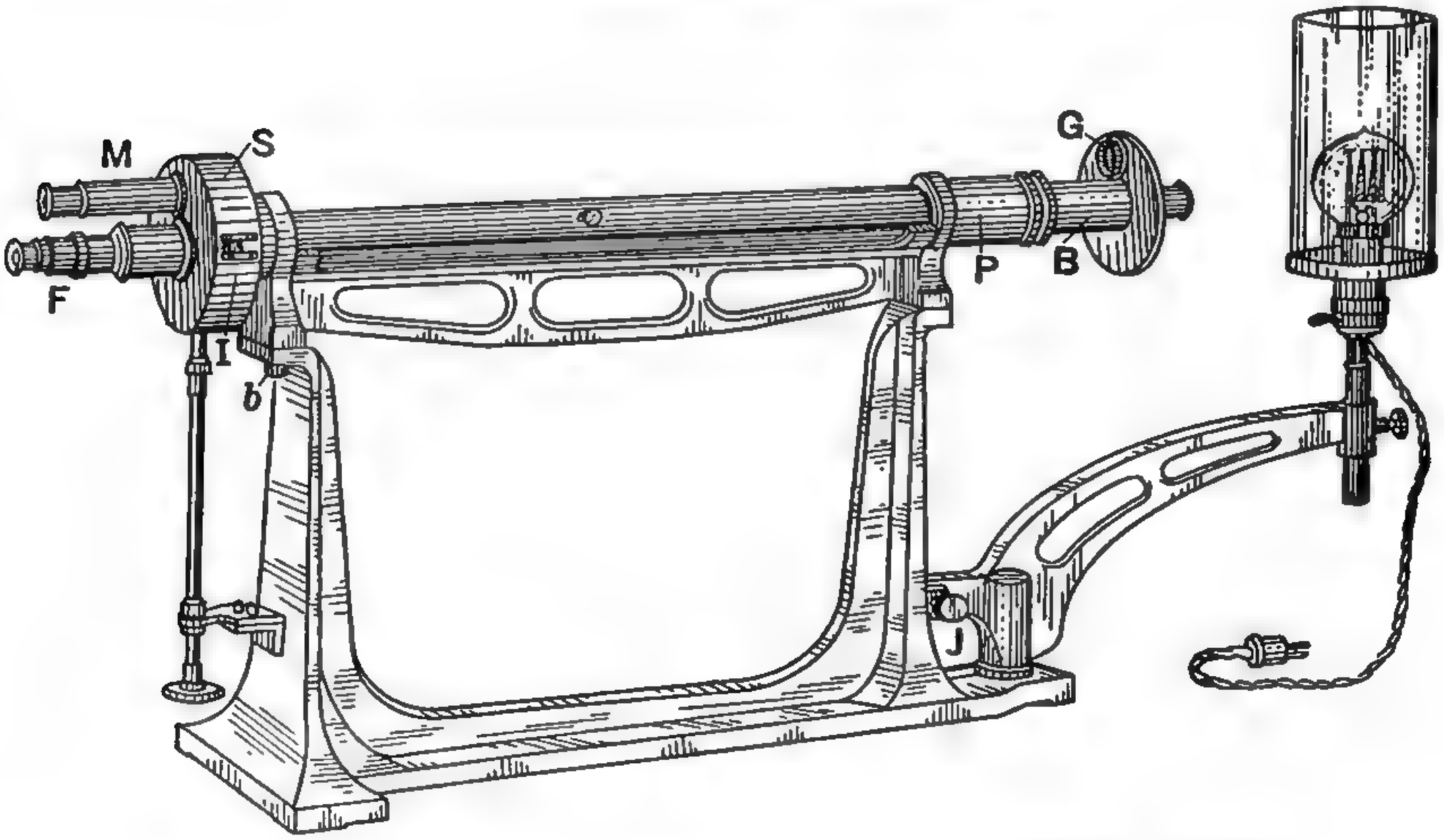
Laurent-Jobin Saccharimeter. The optical arrangement of this instrument (Fig. 118) is practically the same as in the Laurent saccharimeter, but in its mechanical design most of the recommendations of the Committee of the American Chemical Society have been embodied. It is mounted on a trestle support, the trough has a hinged cover, the wedges are protected by an easily removable casing, and the milled screw head controlling the wedge is placed in a convenient position at a short distance above the table top. This saccharimeter is furnished with a scale for a normal weight of 16.269, 20, or 26 g. In

each case the scale is calibrated according to the value adopted by the International Commission in 1936.



(Courtesy of Arthur H. Thomas Co.)

FIG. 118. Laurent-Jobin saccharimeter.



(Courtesy of Akatos, Inc.)

FIG. 119. Schmidt and Haensch single-wedge saccharimeter.

P, position of Lippich polarizer for double or triple field; *S*, casing of sheet brass for protecting wedges from dust; *F*, telescope; *M*, magnifier for reading scale.

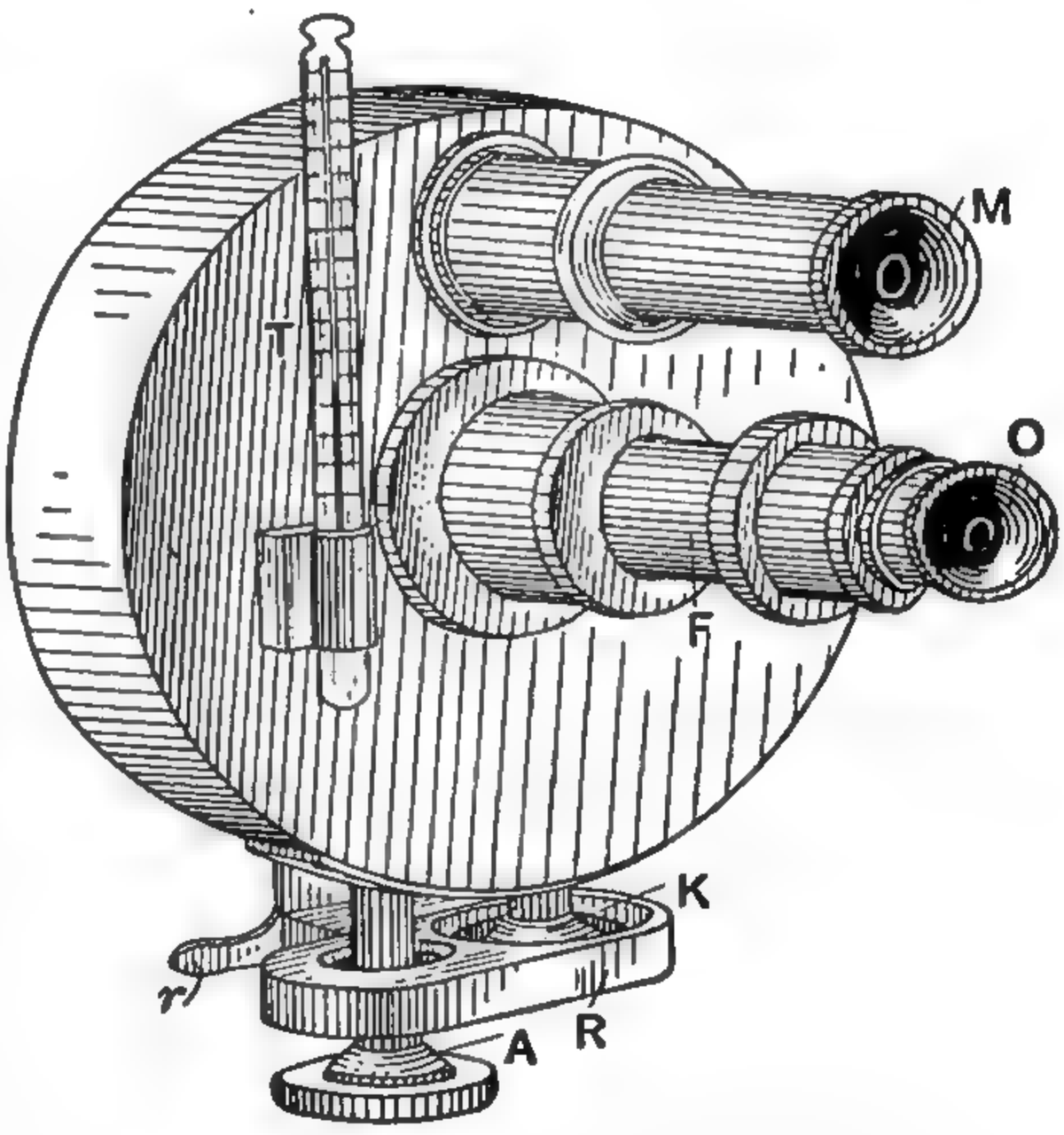
Duboscq-Pellin Saccharimeter. The Duboscq-Pellin saccharimeter for white light, as regards position of polarizer, half-wave plate,

quartz-wedge compensation, etc., is the same as that of the Laurent. The concentric field of the Pellin saccharimeter requires a somewhat different cutting of the half-wave plate, but in other respects the two saccharimeters are very much alike. The later models have the regular double field, divided vertically, and the instruments are equipped, as desired, for a normal weight of 16.269, 20, or 26 g.

The saccharimeter with Lippich polarizer is the form generally preferred. The half-shadow angle between the prisms of the polarizer is usually between 5° and 8°; it can be measured approximately by noting the interval between the points of maximum light extinction each side of the 0 point. The degrees Ventzke between the two points of maximum darkness multiplied by 0.34657 gives the angle of the half shadow.

Schmidt and Haensch Saccharimeters. A single-wedge Schmidt and Haensch saccharimeter upon trestle support is shown in Fig. 119.

The quartz wedge is moved by a milled screw head which is so placed that the hand can rest upon the table during adjustment. The lamp is fixed at exactly the proper distance in a detachable bracket. The same instrument is also furnished with a tripod stand, but this type is not to be recommended, for reasons already explained on p. 200. The reading scale is engraved either on a nickeline plate fastened to the wedge or on the wedge itself. If desired, all Schmidt and Haensch saccharimeters are fitted with a thermometer (see Fig. 120) which indicates the temperature of the quartz wedge or wedges.



(*Courtesy of Akatos, Inc.*)

FIG. 120. Showing front of Schmidt and Haensch double-wedge saccharimeter with thermometer.

The method of scale illumination in Schmidt and Haensch saccharimeters is shown in Fig. 121, which gives the arrangements of parts for a double-wedge instrument. The light from the lamp is focused upon the small window *a* in the wedge housing and is reflected from the mirror *b* through the ground-glass plate *c* upon the scale

from which it is reflected through the prism p into the microscope whose objective is at d and eyepiece at f . . . g. The working wedge is operated by the screw A and the control wedge by the screw K. The

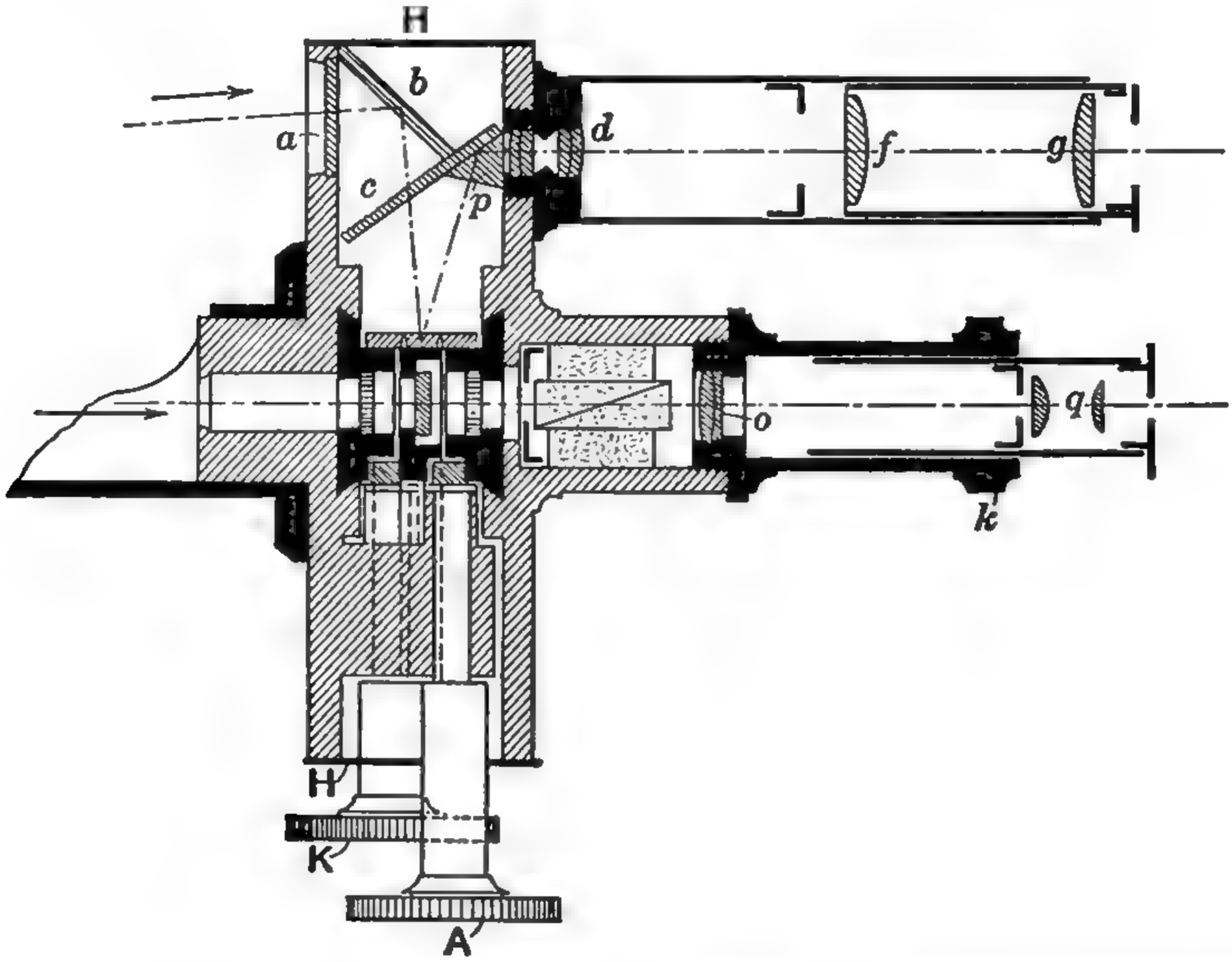


FIG. 121. Device for illuminating scale of Schmidt and Haensch saccharimeter.

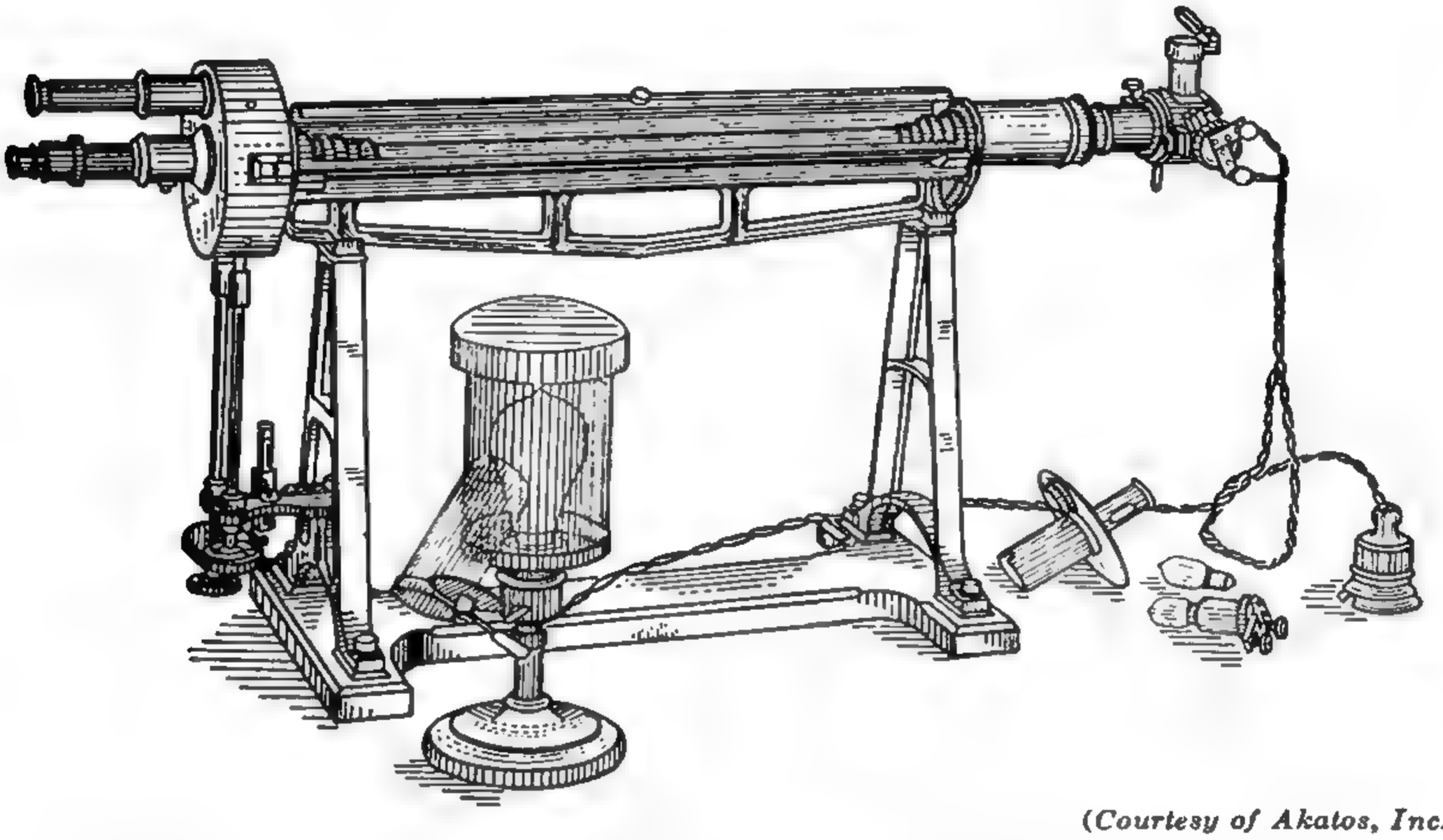
appearance of the scale of this instrument as viewed through the microscope is shown in Fig 110.

An improved type of Schmidt and Haensch saccharimeter is the double-wedge apparatus shown in Fig. 122. The wedges are moved by two milled screw heads one of which is placed at a slightly higher elevation to prevent confusion. After one of the wedges has been set at 0, guard R (shown in detail in Fig. 120) is moved over the milled screw head controlling this wedge; this leaves the other screw head free for moving the wedge on which the reading is taken. This feature prevents disturbing the 0 point after it has once been set.

The special lighting device takes its current directly from a 110-volt circuit; the lamp shown in the foreground of Fig. 122 serves as a resistance and also to illuminate the notebook of the observer. This light can be cut off by moving the shutter over the window through which the light emerges.

Schmidt and Haensch also furnish instruments equipped with a glass filter instead of the bichromate cell. This filter may be moved into or out of the beam of light by means of a small lever.

For use in the tropics, the polarizer and analyzer are mounted so that they can be readily taken out by removing two screws. The openings are then closed with metal plates to keep out the dust. The prisms can be cleaned and kept in a desiccator or drying oven to prevent fungus growth on the prisms while the instrument is not in use.



(*Courtesy of Akatos, Inc.*)

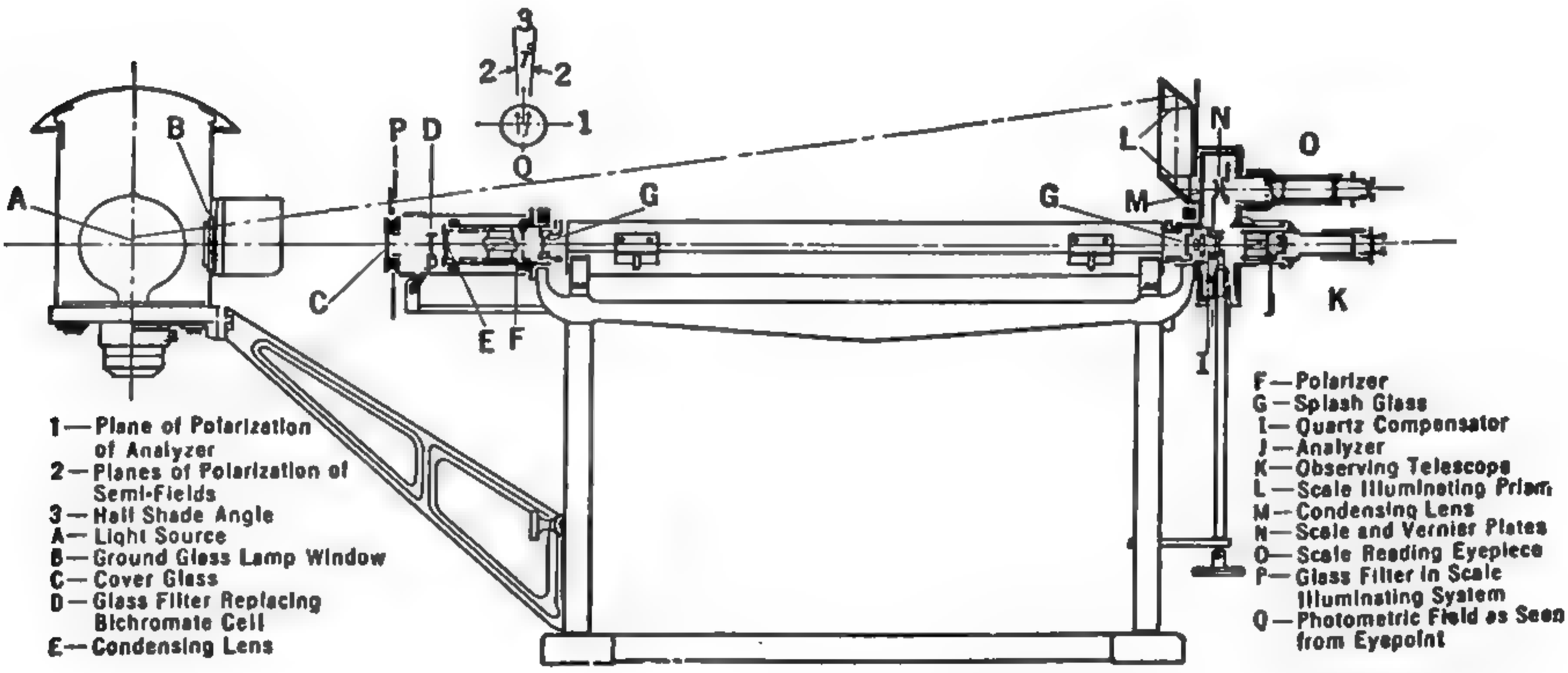
Fig. 122. Schmidt and Haensch double-wedge saccharimeter with electric attachment for illumination.

Frič's Saccharimeter. The half-shadow saccharimeters of J. and J. Frič are very similar in construction to the instruments previously described except in the method of scale illumination. In the latest types of Frič saccharimeter a part of the light, as it passes from the source of illumination through the diaphragm at the end of the instrument, is reflected through a system of mirrors and lenses upon the scales. This illuminating attachment is shown in the Bates saccharimeter (*L* in Fig. 128), but the distinctive feature of the Frič illuminating device is the method of reading the glass scale by transmitted instead of by reflected light. The vertical glass plate, on which the scale is engraved, is mounted upon its quartz wedge and moves directly behind the adjustable vernier plate. The scale divisions, as thus illuminated, appear with great distinctness. There is no troublesome dividing line between scale and vernier, as with metal scales, and readings can be made with the greatest comfort and accuracy (Fig. 111).

Bausch and Lomb's Saccharimeter. In designing this instrument the manufacturers canvassed a large number of sugar and food

chemists in the United States, Cuba, and Hawaii in order to overcome as far as possible certain objectionable features in apparatus of previous construction. The saccharimeter (Fig. 123) embodies most of the points recommended by the Committee of the American Chemical Society and has the following features.

Illumination is provided by a 100-watt concentrated filament tungsten lamp, placed in a well-ventilated housing with a side opening covered by a plate of ground glass. The lamp house is supported by a detachable bracket, fastened to the trestle support of the instrument in such manner that conduction of heat to the polarizer is prevented. The bracket keeps the light always in alignment with the optical system, even if the instrument is moved; the lamp is adjustable vertically and horizontally so that the light can be easily centered.



(Courtesy of Bausch and Lomb Optical Co.)

FIG. 123. Bausch and Lomb saccharimeter.

Between lamp and polarizer is a glass light-filter, which displaces the troublesome cell of bichromate solution ordinarily used. The filter has the same absorptive power as 15 mm. of 6 per cent bichromate solution and may be instantly removed from the field by a push rod, when it is desired to polarize dark solutions. The polarizer is either of the Lippich or Jellett type with a half-shadow angle of about 7°. The analyzer is a Glan-Thompson prism; polarizer and analyzer are so mounted that they can be easily removed and restored to position without disturbing the adjustment.

The quartz compensation is of the single-wedge type and is enclosed in a dust-proof case with removable cover. The wedge is mounted with its scale plate on a carefully fitted slide which is operated without

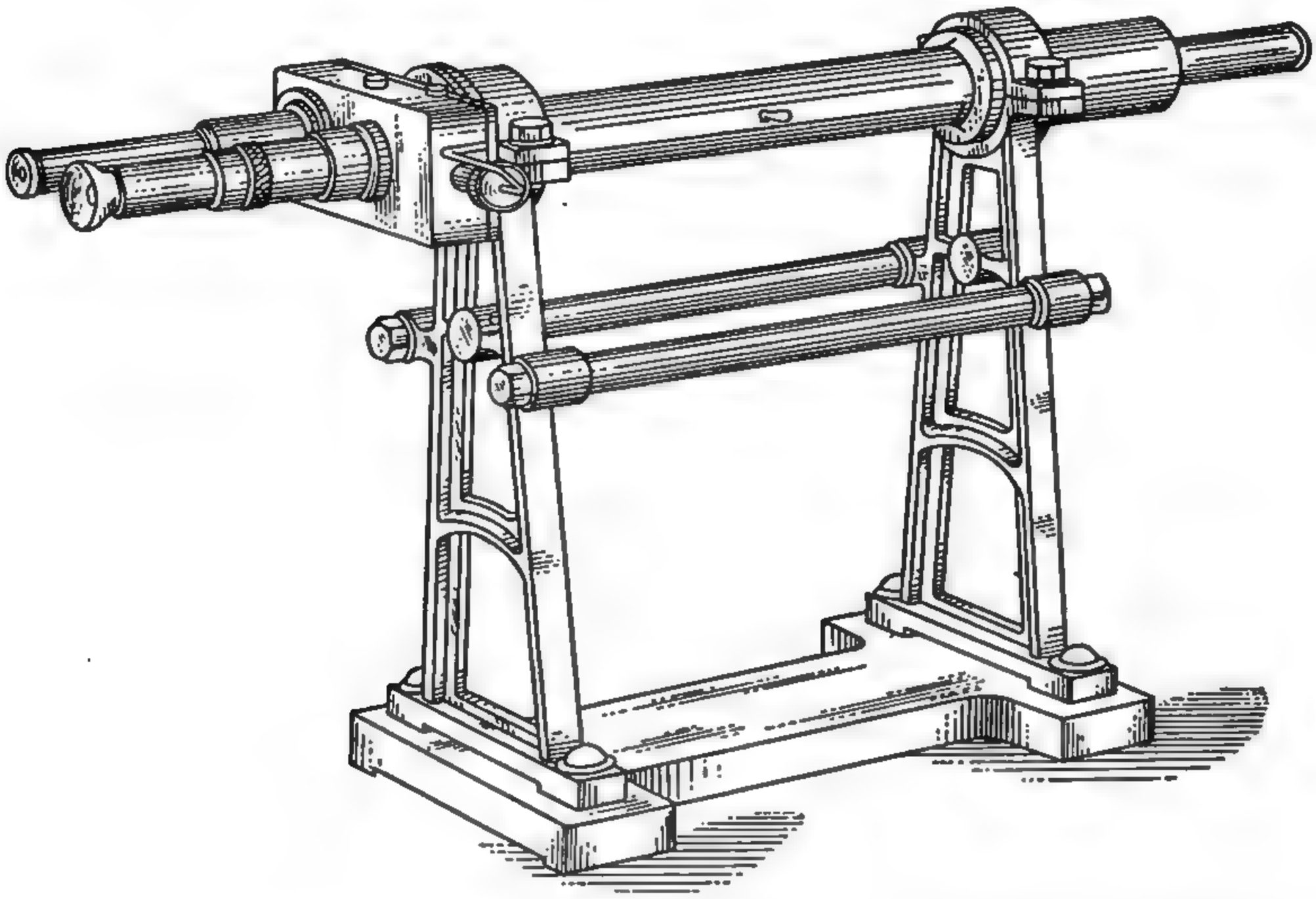
lost motion by a rack and pinion connected with a vertical driving rod. The vernier is adjusted by a differential screw which moves positively in either direction. Scale and vernier are engraved on closely adjacent vertical plates of glass and cover a range of —30° to +110°. For ease in reading, the ends of the scale and vernier lines slightly overlap; this permits the estimation of 0.025 sugar degree with speed and accuracy. The scale is read by transmitted light from the same lamp that illuminates the polarized field, the illumination being so controlled that the scale has about the same depth and color as the polarized field at the end point; this adds to the ease and comfort of reading. Illumination of the scale from the lamp is obtained by two reflecting prisms in such a way that light is not cut off when control or other special tubes are being manipulated.

The trough is V-shaped and made of one piece of metal sufficiently thick to prevent damage under ordinary usage. It is 420 mm. long, so that 400-mm. tubes and control tubes can be accommodated. A slight space between the ends of the trough and the splash-glass holders permits any spilled liquid to drain off without flooding the optical parts of the instrument; this clearance also prevents warping of the trough and transmission of heat to the optical parts when polarizations are made at high temperatures. The trough is removable so that other forms of tube holders and baths may be inserted for special cases. The removable hinged cover with which the trough is provided folds back into a horizontal position, where it may be made a receptacle for tubes. The splash-glasses are of the same size as the tube cover glasses, and their mounts can be easily removed for cleaning or replacing.

Bellingham and Stanley Quartz-Wedge Saccharimeter. This apparatus is shown in Fig. 124. The polarizer used in it has already been described (see p. 159). The entire polarizer unit can be easily removed, cleaned, and put back. In case of necessity it can be replaced by a new one without expert assistance. The quartz compensation system, Fig. 125, is of special design. The movable wedge (4) is circular, and its extension is only 2 cm., which gives greater assurance of homogeneity. According to the manufacturers,[54] the wedge is actually constructed from two opposed wedges of right- and left-rotation quartz placed together in contact to form a parallel plate. Each pair of wedges is mounted in a suitable container or mounting which may be rotated by set screws 2 and 3. If the wedges are rotated through a small angle, the effective angle of the wedge will be altered, and also the lateral movement necessary to compensate a

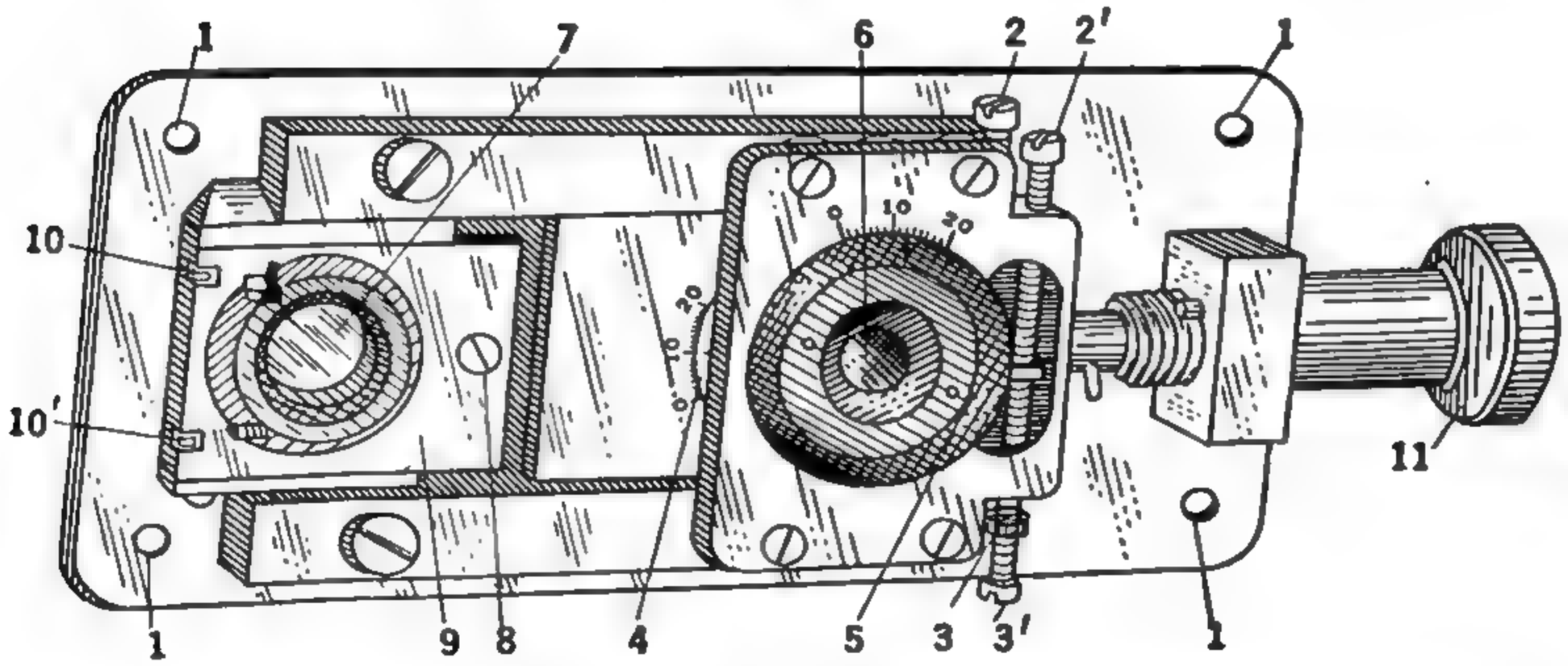
[54] *Intern. Sugar J.*, **24**, 587 (1922).

given solution of sugar. By this means the wedges may be adjusted after the instrument is finally assembled to give the correct scale length to an accuracy of 0.01°. The sugar scale (7) is on glass and is



(Courtesy of Bellingham and Stanley.)

FIG. 124. Bellingham and Stanley saccharimeter.

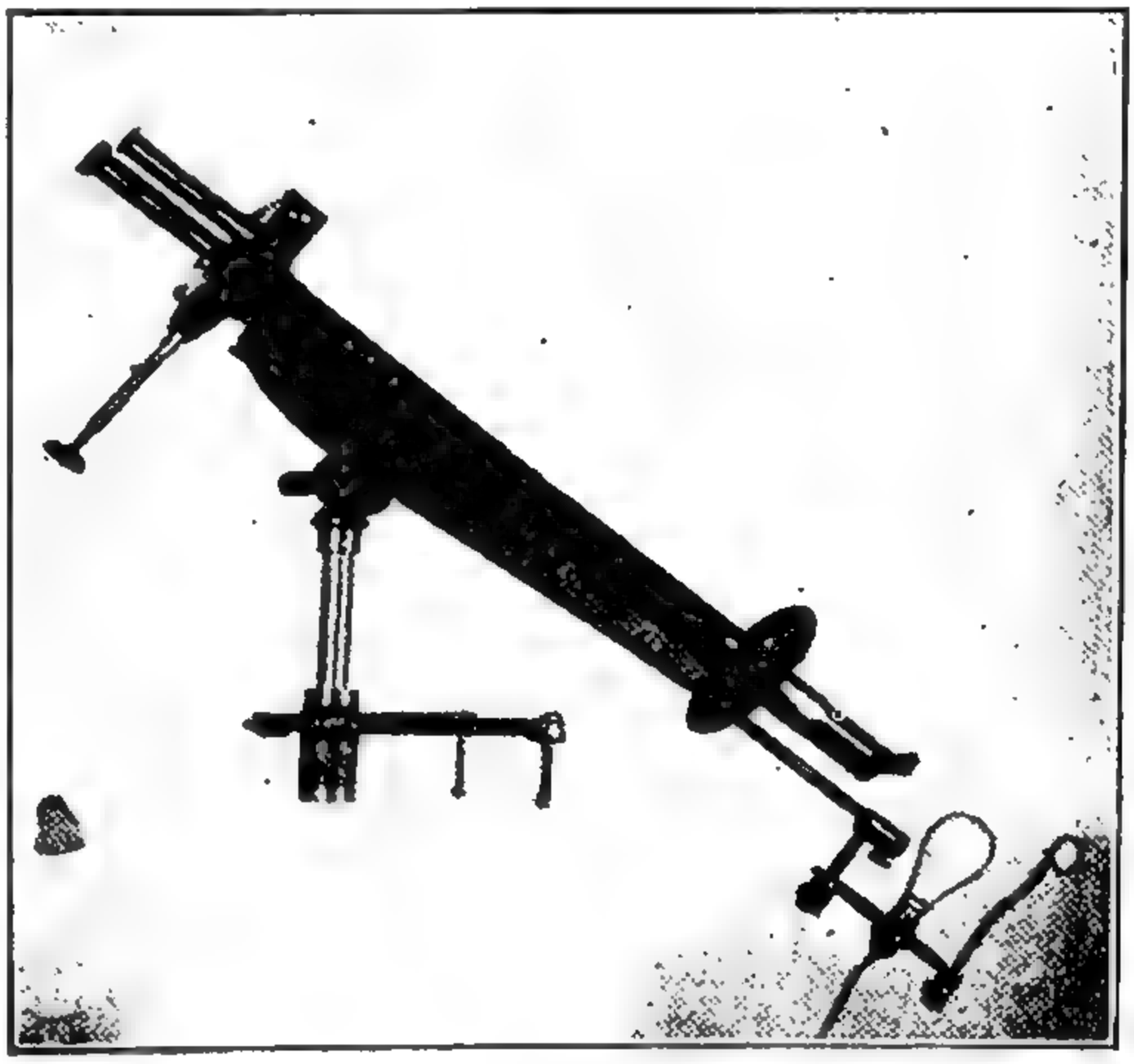


(Courtesy of Bellingham and Stanley.)

FIG. 125. Details of the wedge system in the Bellingham and Stanley saccharimeter.

read by transmitted light through a telescope to the left of the observation telescope. The scale has a range of —30° to +130°. The trough and the trestle stand meet the specifications of the Committee of the American Chemical Society.

Deerr-Darashaw Saccharimeter.[55] This instrument, Fig. 126, is mounted on a heavy pillar which passes through a horseshoe-shaped base, and can be raised and lowered. The saccharimeter proper is attached to the pillar by a heavy girder with a swivel joint by which it can be placed either in the usual horizontal position or at an angle, so that the observer may assume a comfortable posture, looking downward into the telescope. In this case a hole is cut in the table, and the horseshoe base is fastened to a shelf below the table top. The lamp is fixed on a bracket attached to the instrument, and there is also a small mirror to illuminate the scale.



(*Courtesy of Mr. Noel Deerr.*)

FIG. 126. Deerr-Darashaw saccharimeter.

The polarizer and analyzer are of the same type as in the Bellingham and Stanley saccharimeter. Each of them is mounted in a short tube, closed at both ends with cover glasses in screw caps. A tongue is attached to the under side of the prism box, passes through a slot in the barrel of the instrument at either end, and is held in position by two screws running through small brackets on opposite sides. This makes it possible to rotate both polarizer and analyzer through a small angle and to fix them in the desired position by tightening the screws. Both prisms can be readily taken out for cleaning, by loosening one of the screws, removing the telescope tube at the front end and the lens holder at the rear end, and simply sliding the prism boxes out. The quartz-wedge system is also mounted in such a way that all parts are easily accessible, and that the compensator plate, the fixed wedge, the sliding wedge, and the vernier scale can be removed, without trouble, cleaned, and put back in place. The wedge scale is attached to one side of the sliding wedge, and not fixed on its surface, as in other instruments. Both scales are engraved on glass, and the vernier is located behind the wedge scale; it can be adjusted so that the scale marks just touch, or overlap, as desired. The splash-glasses

[55] *Intern. Sugar J.*, **35**, 138, 432 (1933).

are mounted in sliding holders which pass through slots at either end of the trough, so that they can be cleaned easily. The bichromate cell is replaced by a gelatin filter. Near the end of the instrument is another sliding holder, moving in a slot and having three apertures side by side, one for the gelatin filter, one for a clear-glass disk, and the third for a frosted-glass disk, so that any of them may be inserted between the lamp and the polarizer.

Quartz-Wedge Saccharimeters with Variable Sensibility

Of the instruments previously described, the French saccharimeters, using a Laurent half-wave plate and employing monochromatic or bichromate-filtered white light, are the only forms of apparatus which permit a variation of the half-shadow angle to suit the requirements of greatest sensibility.

In all the Schmidt and Haensch saccharimeters the half-shadow angle is fixed. An attachment for shifting the large prism of the Lippich polarizer and regulating the half-shadow angle has been supplied by some manufacturers, as in the double quartz-wedge saccharimeter of Peters.[56] But since a change in the half-shadow angle produces a change in the 0 point it is necessary to provide for rotation of the analyzer also.

The Jobin and Yvon Saccharimeter. A more recent instrument based on this principle, but with a single quartz wedge, has been designed by Jobin and Yvon. It is equipped with a Lippich polarizer the half-shadow angle of which can be varied. In order to correct for the ensuing change in the 0 point, the vernier is set at 0 and the analyzer is rotated until the two halves of the field show equal brightness. When this adjustment has been made the two halves of the field usually have a difference in color. The analyzer is then rotated slightly to darken the reddish half of the field, and equality in brightness is restored by slightly shifting the movable quartz wedge. These operations are repeated until the two halves of the field are evenly matched in both brightness and tint. The setting is verified by several check readings, and the adjustment must be made with great care.

Saccharimeters of this type have been condemned by Landolt[57] on the ground that the delicate manipulations required may readily lead to considerable error when such instruments are used in the factory.

Bates's Saccharimeter. To obviate the objection last named, Bates[58] has devised an attachment which rotates the analyzer automati-

[56] *Z. Ver. deut. Zucker-Ind.*, **44**, 221 (1894).

[57] " Das optische Drehungsvermögen," 2nd ed., p. 351, 1898.

[58] *U. S. Bur. Stand. Bull.*, **4**, 461; *Z. Ver. deut. Zucker-Ind.*, **58**, 105 (1908).

cally and makes it possible to correct the 0-point error for any change in the half-shadow angle without resetting the scale. The principle of the Bates saccharimeter can be understood from Fig. 127.

Let *OP* be the direction of the plane of the large Nicol and *ON* that of the small Nicol in a Lippich polarizer; let *AZ* be the plane of the analyzer at right angles to *OB*, the bisection of the half-shadow angle *PON* or α. We will suppose for a moment that the intensities of light in *OP* and *ON* are equal and that the plane of the large Nicol is moved

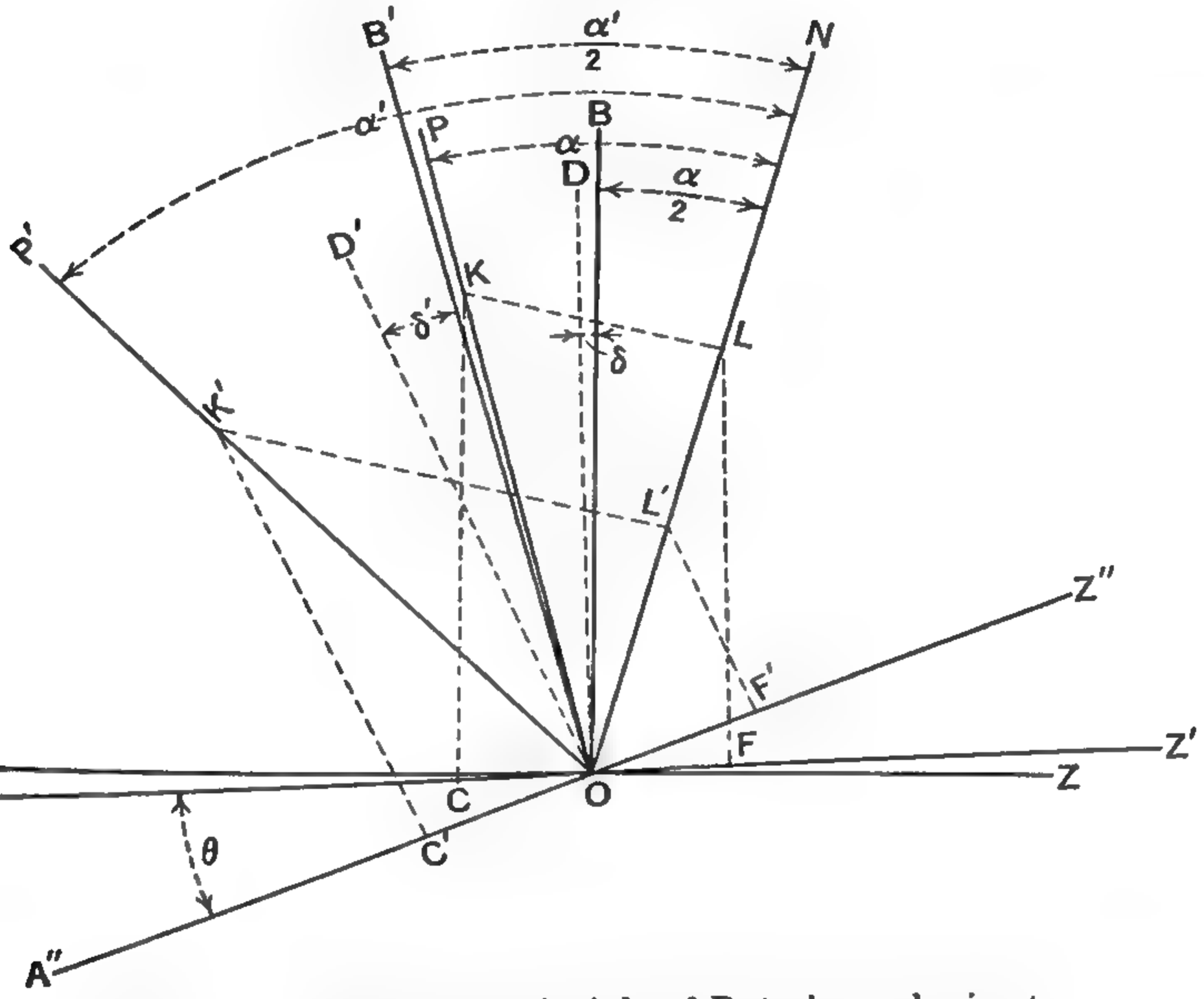


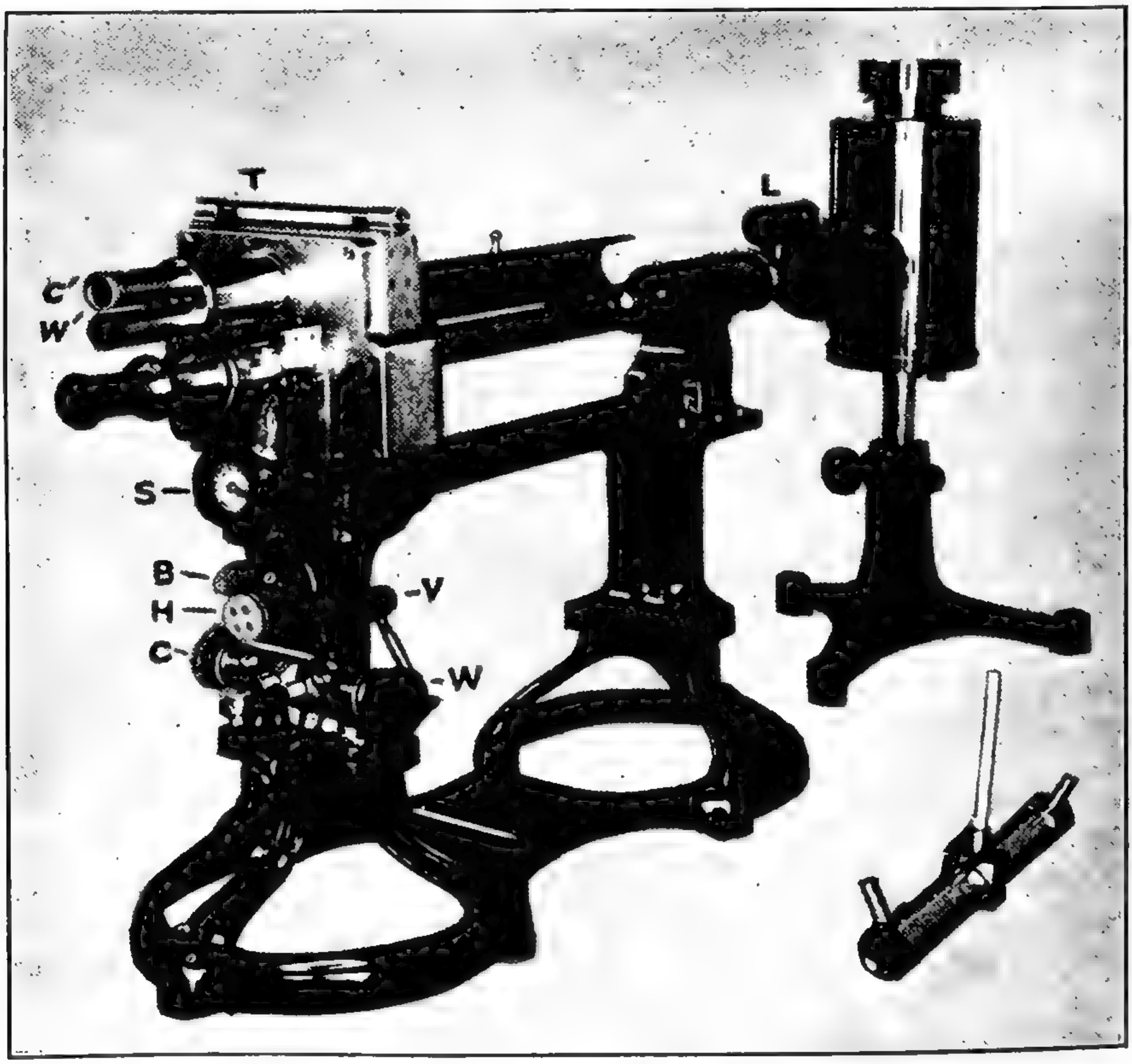
FIG. 127. Illustrating principle of Bates's saccharimeter.

from *OP* to *OP'*, forming with the plane of the small Nicol the new angle *P'ON* or α'. To obtain uniformity of field at the 0 point for the new angle α' the bisection *OB* must be moved to *OB'*. It will be seen from the diagram that the angle $BOB' = \frac{\alpha'}{2} - \frac{\alpha}{2} = \frac{\alpha' - \alpha}{2} = \frac{POP'}{2}$. To correct, therefore, for the displacement of 0 point, assuming the intensities of light to be always the same for both Nicols, the plane of the analyzer must be moved through one-half the angular displacement of the large Nicol of the polarizer.

In the Lippich system, however, the intensities of light are not equal for the large and small prisms of the polarizer. A part of the light is

extinguished in the small Nicol and there is also a loss from reflection and absorption. We will consider first the light lost by absorption.

Let OK = amplitude of light from large Nicol. Draw $KL \perp ON$; then OL = amplitude of light from small Nicol; the plane of the ana-



(Courtesy of National Bureau of Standards.)

FIG. 128. Bates's saccharimeter with variable sensibility.

W, milled head for operating working wedge; *V*, auxiliary screw for fine adjustment of *W*; *C*, milled head for operating control wedge; *B*, auxiliary screw for fine adjustment of *C*; *W'*, microscope for reading working wedge scale; *C'*, microscope for reading control wedge scale; *S*, scale indicating " degrees of brightness " or half-shadow angle.

lyzer AZ must then be removed to $A'Z'$ so that the amplitudes OC and OF will be equal in each half of the field. The angles AOA' and BOD, through which the plane of the analyzer and its perpendicular have moved, is δ or the change from the true zero point when the intensities of light in OP and ON are equal, in which case $\alpha = 0$.

We will suppose in order to increase the intensity of light for the half shadow that the plane OP of the large Nicol is moved to OP', increasing α to α'. The amplitude OK' remains the same as OK. Draw $K'L' \perp ON$; then the amplitude in $ON = OL'$. The plane of the analyzer must now be moved to $A''Z''$ in order that the $\perp^s$ $K'C'$ and $L'F'$ cut off the equal amplitudes OC' and OF' in the two halves of the field. OD' which is $\perp$ $A''Z''$ will then form, with OB', the bisection of α', the new angle δ'. The angle $\theta = DOD'$ through which the analyzer has moved from its previous position is expressed by the equation

$$\theta = \delta' + \frac{\alpha' - \alpha}{2} - \delta \cdot$$

In the polariscope of Bates (Fig. 128) the analyzing Nicol and the large Nicol of the polarizing system are mounted in bearings and are joined by gears with a connecting rod. The milled head which operates the driving mechanism is shown at H. When the milled head is turned the two Nicols are rotated and the design of the gears is such that the analyzing Nicol always receives one-half the angular displacement of the large Nicol of the polarizing system. Above the milled head is a circular scale which shows the polarizing angle for any position of the Nicols. In moving the plane of the large polarizing Nicol through the angle POP' (Fig. 127) the rotating device of Bates's polariscope moves the plane of the analyzer through the angle BOB'. In this way the zero-point error of the instrument will always be equal to the value of δ for any angle of the half shadow, assuming that the zero has been previously adjusted for $\alpha = 0$. If the zero point of the instrument is set for any value of the half shadow α, and α is then changed to α', the zero will have an error of $\delta' - \delta$ (the analyzer having rotated $\frac{\alpha' - \alpha}{2}$, this value disappears from the equation

$$\theta = \delta' + \frac{\alpha' - \alpha}{2} - \delta\Big)$$

The calculated values of δ in Ventzke degrees for different values of the half-shadow angle α according to the two equations,

$$\tan \delta = \tan^3 \frac{\alpha}{2} \quad \text{and} \quad \tan \delta = \frac{1 - \cos \alpha \sqrt{0.92}}{1 + \cos \alpha \sqrt{0.92}} \tan \frac{\alpha}{2}$$

(see p. 156), are given in Table XXXVI.

The values of δ in the second column are greater than those in the first column by 0.03 α. The true values of δ according to Bates lie between those calculated by the two equations and will vary according

TABLE XXXVI

CALCULATED VALUES OF ERROR IN 0 POINT FOR BATES'S SACCHARIMETER

Values of δ in Ventzke degrees

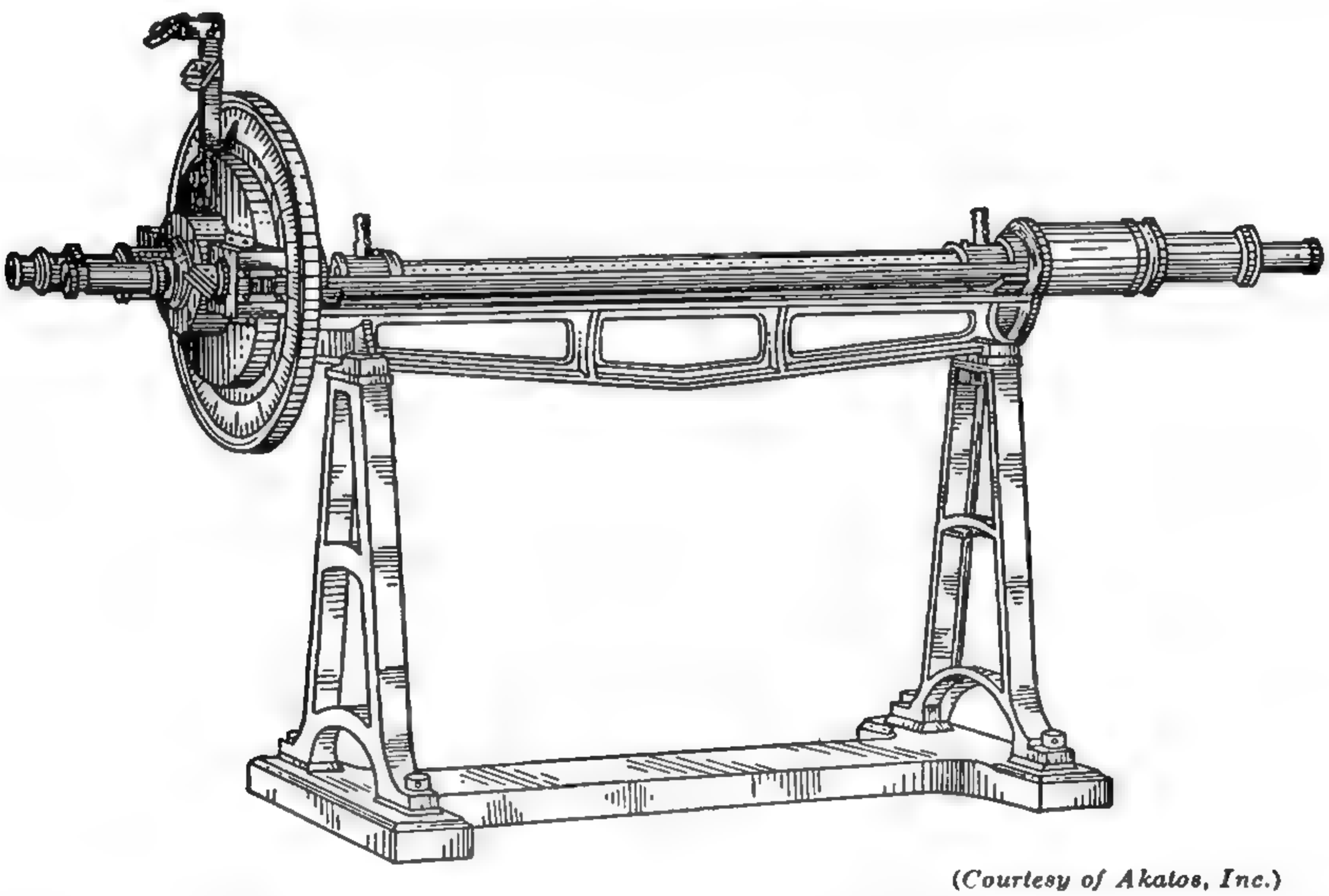
Values of α circular degrees	I By Formula $\tan \delta = \tan^3 \frac{\alpha}{2}$	II By Formula $\tan \delta = \frac{1 - \cos \alpha\sqrt{0.92}}{1 + \cos \alpha\sqrt{0.92}} \tan \frac{\alpha}{2}$
1°	0.003	0.033
2	0.004	0.064
3	0.005	0.096
4	0.008	0.129
5	0.014	0.164
6	0.024	0.205
7	0.038	0.249
8	0.057	0.299
9	0.080	0.352
10	0.110	0.412
11	0.150	0.482
12	0.192	0.554

to the construction of the instrument. This true value of δ will be the value by the first formula $\pm c\,\alpha$ in which c is a constant for each individual Lippich system. If a Bates saccharimeter is set, therefore, for $\alpha = 0$, the calculated change in zero point for variations in α can be easily applied to the scale reading. If the instrument is set for any particular value of α, as 8°, the half-shadow angle may be increased or diminished several degrees from this point without introducing a change in 0 greater than 0.1° V. or S.

The Bates saccharimeter, constructed by Josef and Jan Frič of Prague, is the standard instrument of the United States Customs Service. The apparatus presents several advantages over the ordinary saccharimeter, but the mechanical difficulties of construction make it expensive. The instrument is not provided with a bichromate light filter. Though this omission may occasion no serious error in the polarization of colored solutions (as of low-grade sugar-house products), a bichromate light filter is required in the examination of high-grade cane sugars, starch-conversion products, and many other substances. An absorption cell for this purpose should be placed just in front of the aperture between the saccharimeter and the source of light. A very commendable feature of the Bates instrument is the thermometer (*T*, Fig. 128) which indicates the temperature of the quartz wedges. The milled heads, *C* and *W*, for setting the field, can be locked in position by small levers, and are provided, in the later models of the instrument, with auxiliary screws, *B* and *V*, for fine adjustment.

Saccharimeters with Magnified Scale

For special kinds of work involving the investigation of products with a narrow range in composition, saccharimeters have been constructed with a limited magnified scale. The saccharimeter shown in Fig. 129 is a modern modification of an instrument originally devised by Stammer[59] for polarization of sugar beets. In this apparatus a magnified scale, reading from 0° to 35° V., is engraved on a large circle which is rotated by hand, and which is connected through a worm drive



(Courtesy of Akatos, Inc.)

FIG. 129. Stammer saccharimeter with magnified scale for polarizing sugar beets.

with the quartz wedge. The wedge also carries the usual scale, graduated from 0° to 35°, and agreeing exactly with the magnified scale. The 0 point of both scales can be adjusted, that of the magnified scale by moving the pointer slightly to the right or left. Both graduations are illuminated by mirrors, and the large circular scale can be read to 0.1° with the unaided eye.

Saccharimeters of the above type are especially adapted for the polarization of mother beets for seed production; they are constructed for tubes of 200-mm., 400-mm., and 600-mm. length.

[59] *Z. Ver. deut. Zucker-Ind.*, 37, 474 (1887).

Instruments with a magnified limited scale will be found to relieve eye fatigue, where large numbers of analyses of a single product have to be performed. With one person to prepare the tubes of sugar solutions, a second to manipulate the saccharimeter, and a third to note the readings, a large number of polarizations can be made in a very short period of time.

Bachler Tare Room Saccharimeter. The above apparatus has been further improved by Bachler[60] in such a way that the setting of the field and the reading of the scale can be checked simultaneously by a second observer. The instrument is shown in Fig. 130, and in Fig. 131 the special observation device in which the light passes from the analyzer into a Lummer-Brodhun prism (see p. 628). A part of the beam is transmitted through this prism into the regular telescope; another part is reflected into a second telescope at right angle to the other. The setting of the field by one observer can thus be verified at the same time by a second observer. The magnified scale is engraved not only on the front of the disk near its edge, but also on its outer periphery, to be read independently by the second observer. A system of mirrors throws the light from the upper opening of the lamp on the quartz-wedge scale and on the two magnified scales. The light from the lower opening of the lamp passes through a metal tube directly into the polarizing system of the saccharimeter. Two thermometers are furnished with the instrument, one to determine the temperature of the quartz wedge, and the other that of the solution. This saccharimeter is made only for tubes 400 mm. long. It is especially valuable for the analysis of beets by representatives of the buyer and seller, because it automatically eliminates disputes over the sugar content.

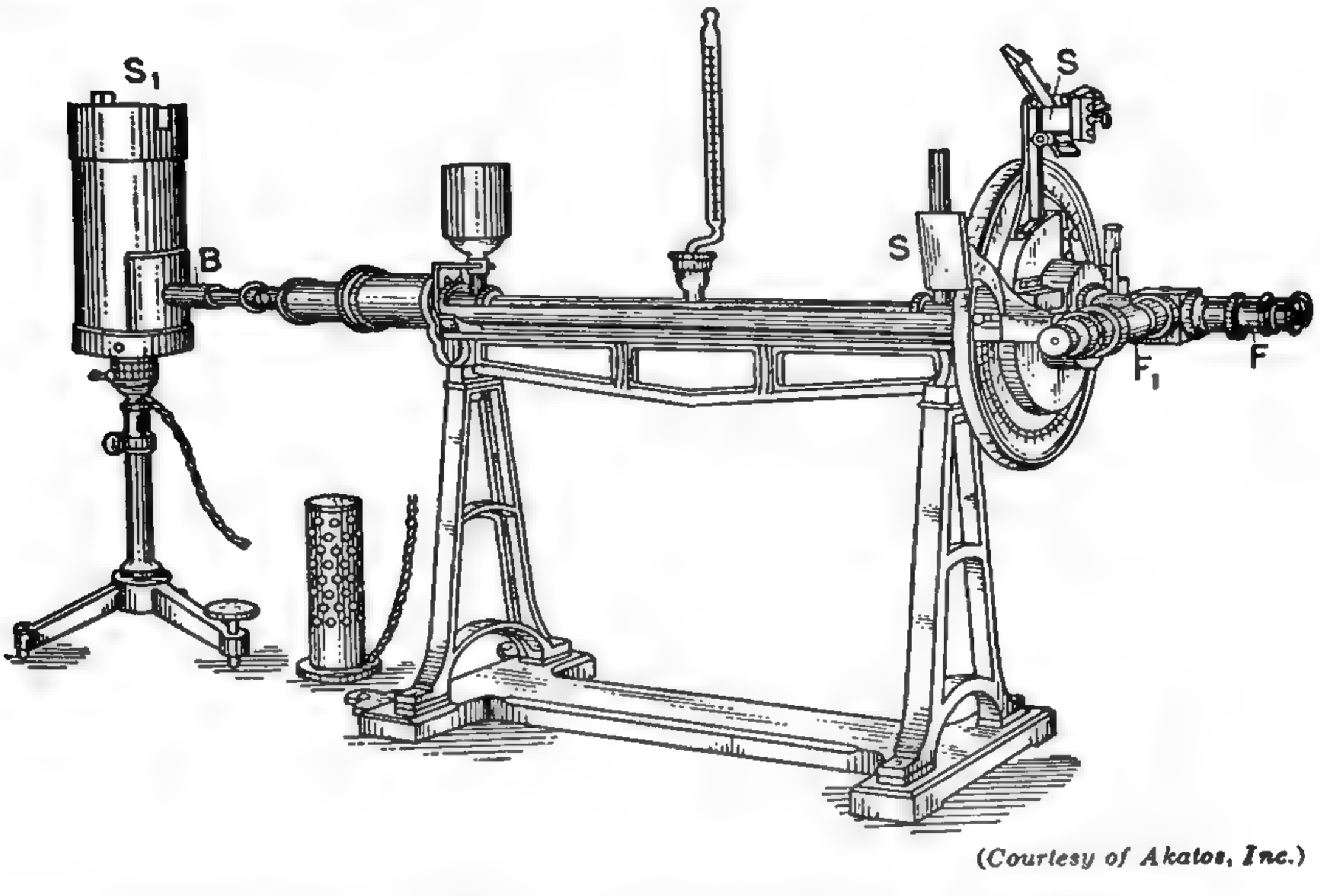
Saccharimeters similar to the above instruments have been constructed with a magnified scale on the quartz wedge, reading between 80 and 100, for the polarization of sugars. These are manufactured usually only for use with tubes 400 mm. long, and they employ a normal weight of 26 g. to 100 ml. solution. Doubling the length of the observation tube necessitates of course doubling the interval between the scale divisions and thus facilitates the reading.

Saccharimeters without Quartz-Wedge Compensation

It has been mentioned on p. 168 that the development of the electric sodium-vapor lamp has made it possible to dispense with the quartz-wedge compensation and to use instead polarimeters with a cane-sugar scale. These instruments have the advantage of lower cost

[60] *Facts About Sugar*, **16**, 337 (1923).

and at the same time greater accuracy because perfectly homogeneous quartz wedges are difficult to procure.



(*Courtesy of Akatos, Inc.*)

Fig. 130. Bachler's tare room saccharimeter for two observers.

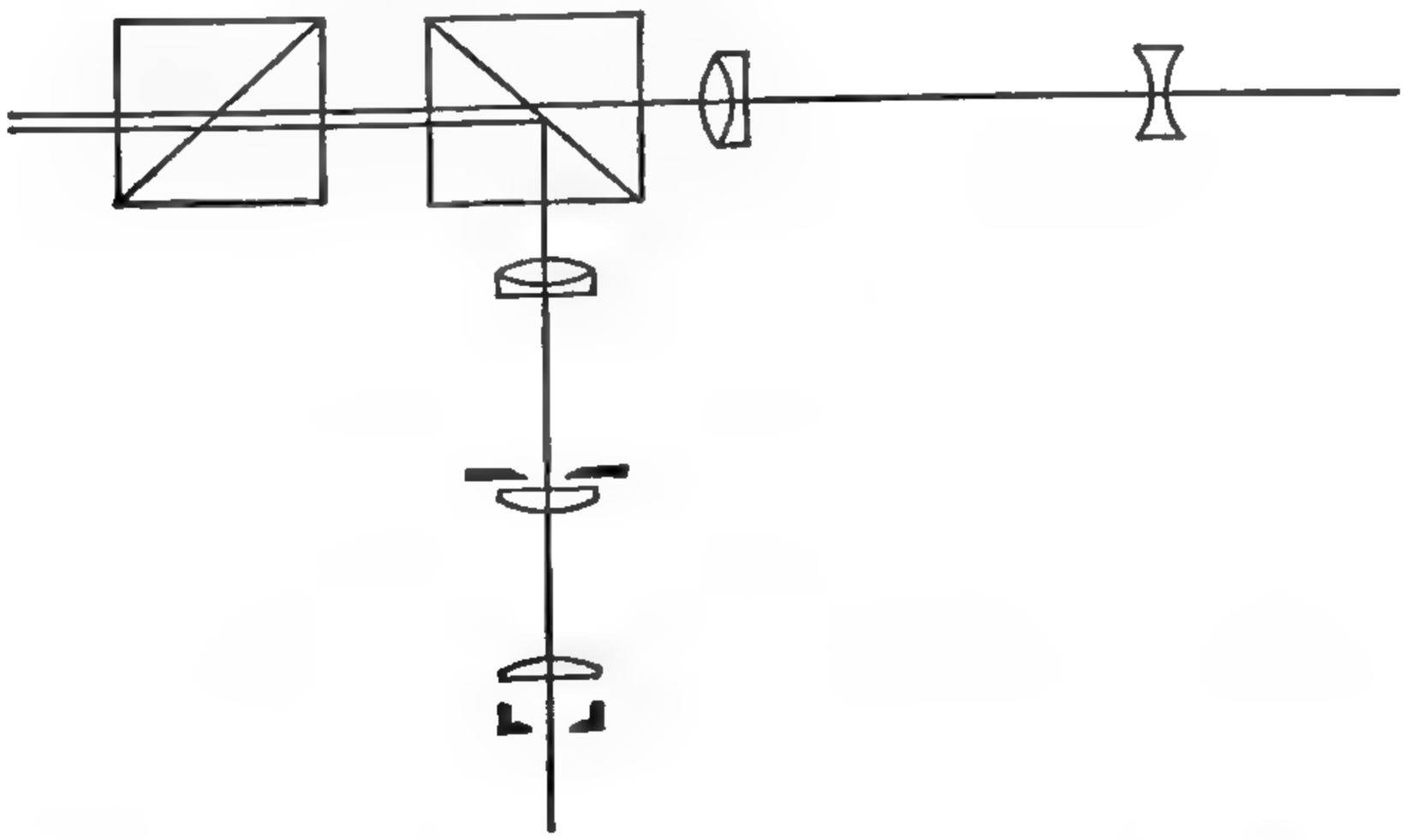
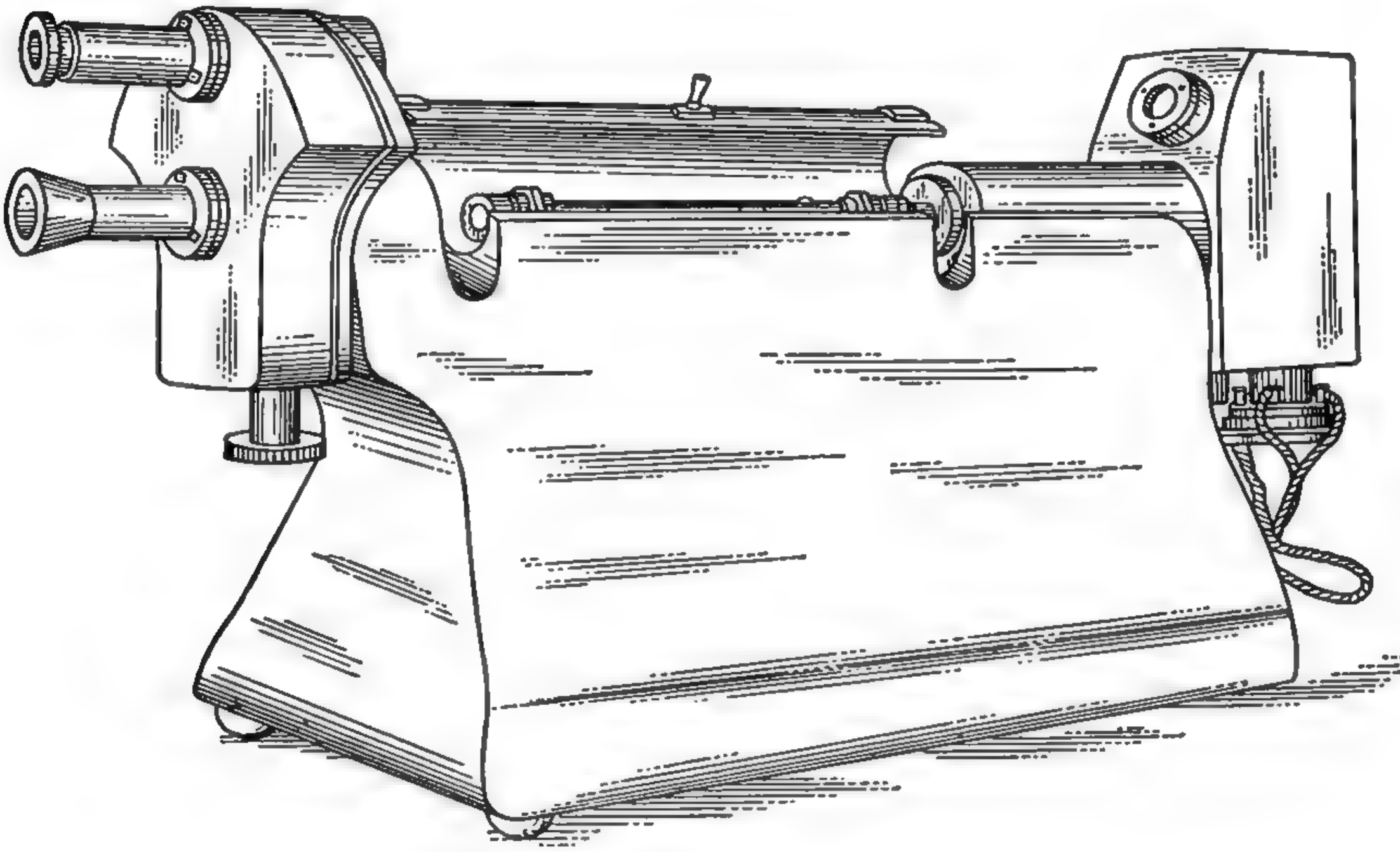


Fig. 131. Optical arrangement of Bachler's reading device for two observers.

The Bellingham and Stanley Polarimeter-Saccharimeter.[61] This polarimeter, specially designed for routine sugar analysis, is shown in

[61] *Intern. Sugar J.*, 41, 349 (1939).

Fig. 132*a*. It is mounted on a heavy stand with curved surfaces, to reduce the accumulation of dust and spilled liquid to a minimum. The electric sodium-vapor lamp forms an integral part of the apparatus; its housing is open at the rear, to make necessary adjustments. The instrument has the usual polarizer and analyzer system. The analyzer is rotated by means of a milled screw head. The sugar scale, calibrated according to the Bates-Jackson value (100 S. = 34.62 circular degrees), is etched directly on the glass circle, and the 0 point can be readily adjusted. The scale is read by transmitted light, obtained from the



(Courtesy of Bellingham and Stanley.)

FIG. 132*a*. Bellingham and Stanley saccharimeter without quartz-wedge compensation.

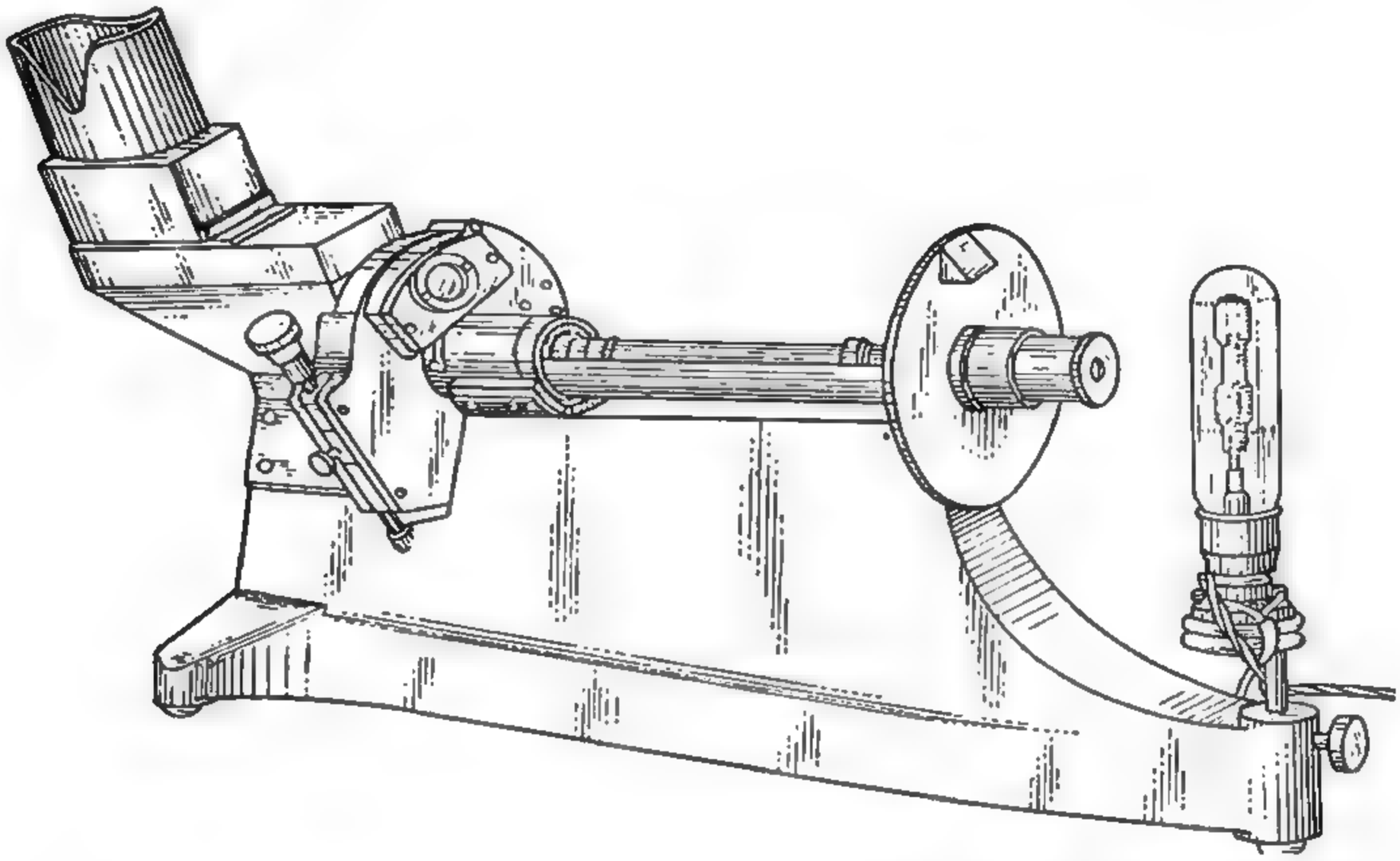
sodium lamp by means of a deflecting prism. The splash-glasses are not placed at the ends of the tube trough, but are separated from it by deep grooves; this makes them more readily accessible and easier to clean. The limit of accuracy is approximately 0.02° S.

Polarimeters with a cane-sugar scale, to be used in connection with an electric sodium-vapor lamp, are manufactured also by Hilger in England, by Jobin and Yvon in France, by Schmidt and Haensch, and by others.

The Bellinghar and Stanley Projection Saccharimeter.[62] In this instrument, Fig. 132*b*, the principle employed in the projection re-

[62] *Intern. Sugar J.*, 40, 433 (1938).

fractometer (see p. 128) is applied to the readings. Another novel feature has been introduced by placing the observation end at a right angle to the optical axis. The trough is thus more conveniently located for changing tubes. The apparatus is equipped with a glass circle upon which the sugar scale (Bates-Jackson) is engraved, reading from —30° to +100°. The vernier is etched on a separate piece of



(*Courtesy of Bellingham and Stanley.*)

FIG. 132*b*. Bellingham and Stanley projection saccharimeter without quartz-wedge compensation.

glass. The circle is turned by hand, and fine adjustment is made by means of a screw. The observations are made through a hood at the left end of the instrument, no dark closet being necessary. The optical field is seen on the right-hand side, and the scale and vernier are projected to the left side. The setting of the field and the readings are made with the naked eye. The scale can be read to 0.01° S.

CONVERSION FACTORS FOR POLARISCOPE AND SACCHARIMETER SCALES

In the following table factors are given for converting 1° of the various polariscope scales into its equivalent in circular degrees, or in degrees of the different saccharimetric scales. The conversion factors are usually calculated from the angular rotation in purified sodium light produced by a quartz plate which reads 100 sugar degrees in a saccharimeter with bichromate-filtered white light. Bates and Jackson have stated that the angular rotation, for the D line, of the normal

sugar solution (34.617°) is, within the limits of experimental error, the same as that of the normal quartz plate (34.620°), although Schönrock had reported a slightly higher figure for sucrose (34.667°) than for quartz (34.657°).

Scale		Equivalent	
1° V. (Herzfeld-Schönrock)	= 0.34657°		angular rotation, D line
1°	angular rotation, D line	= 2.88542° V.	(Herzfeld-Schönrock)
1° S. (Intern. Commission)	= 0.34620°		angular rotation, D line
1°	angular rotation, D line	= 2.88850° S.	(Intern. Commission)
1°	French sugar scale	= 0.21667°	angular rotation, D line
1°	angular rotation, D line	= 4.61531°	French sugar scale
1°	French sugar scale	= 0.62518° V.	(Herzfeld-Schönrock)
1° V. (Herzfeld-Schönrock)	= 1.59953°		French sugar scale
1°	French sugar scale	= 0.62585° S.	(Intern. Commission)
1° S. (Intern. Commission)	= 1.59782°		French sugar scale
1°	20-g. sugar scale	= 0.26636°	angular rotation, D line*
1°	angular rotation, D line	= 3.75432°	20-g. sugar scale*
1°	20-g. sugar scale*	= 0.76856° V.	(Herzfeld-Schönrock)
1° V. (Herzfeld-Schönrock)	= 1.30113°		20-g. sugar scale*
1°	20-g. sugar scale*	= 0.76938° S.	(Intern. Commission)
1° S. (Intern. Commission)	= 1.29974°		20-g. sugar scale*
1°	20-g. sugar scale*	= 1.22933°	French sugar scale
1°	French sugar scale	= 0.81345°	20-g. sugar scale*

* Correct value according to Baissac (see p. 181).

For the yellow-green mercury line, 546.1 mμ, there is a considerable difference between the conversion factor for the normal quartz plate and for the normal sugar solution, as is shown in the table on p. 183.

CHAPTER VII

POLARISCOPE ACCESSORIES

ILLUMINATION OF POLARISCOPES

For the illumination of polariscopes and saccharimeters numerous lamps have been devised, and the chemist must be guided in his selection by type of instrument, nature of substance to be polarized, and the kind of light supply available. Before the various types of lamps are described a word should be said regarding the general subject of illumination.

A much-neglected point in the illumination of polariscopes and saccharimeters is the placing of the light at the proper distance from

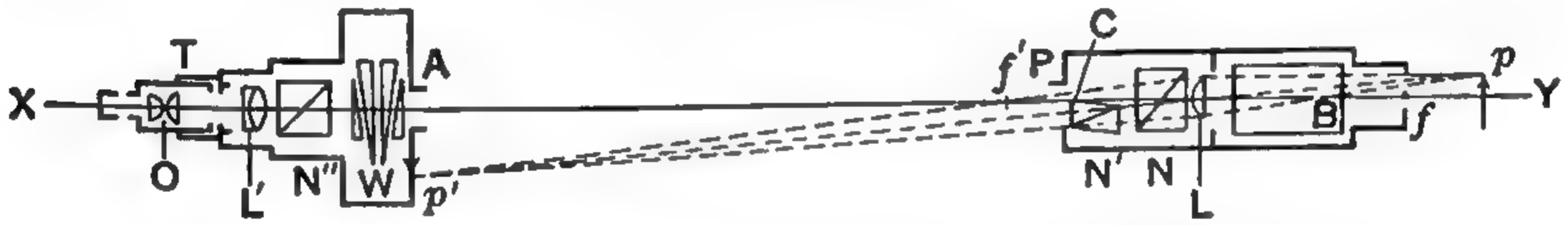


FIG. 133. Showing method of illuminating polariscopes.

the condensing lens. The light should never be placed so near as to overheat the metal at the end of the instrument; neglect of this precaution may cause a softening of the balsam and wax mountings of the polarizer and lead to serious derangement of the optical parts.

The proper rule in setting up the polariscope is to place the light in such a position that its image is clearly defined upon the analyzer diaphragm; this is best accomplished by fastening a needle or other sharp-pointed object just before the light and moving the instrument or light until a clear inverted image of the point is obtained upon a piece of white paper placed before the analyzer diaphragm. When the light is thus focused the polariscope is least susceptible to changes in zero point. The proper position of polariscope with reference to light can be seen from Fig. 133, which shows the arrangement of the optical parts in a double-wedge saccharimeter. When the instrument is correctly placed an inverted magnified image of the light (arrow at p) is obtained at A. The reciprocal of the focal distance of the condensing lens will then equal the sum of the reciprocals of the distances of lens from light and of lens from image.

Example. In a Schmidt and Haensch saccharimeter the focal distance of the condensing lens was found to be 5 inches; the distance from lens to analyzer diaphragm was 20 inches; the distance x for placing the light would then be found from the equation: $\frac{1}{x} + \frac{1}{20} = \frac{1}{5}$; $x = 6\frac{2}{3}$ inches from the condensing lens.

The telescope T (Fig. 133) is focused by the observer upon the dividing line of the field at C, and the analyzer or compensator is turned to the point of even illumination. The dividing line at C will then disappear and the entire field appear of equal intensity. This will occur even with slight variations in intensity in different parts of the illumination, since at the point C, upon which the eye of the observer is focused, the light from any part p of the illumination will be dispersed through different parts of the field (as shown in the figure by the dotted lines); any slight unevenness in the source of illumination will thus be distributed and not noticed by the eye. Great irregularities in illumination, however, must be avoided, and for this reason it is important that the instrument be kept in perfect alignment with its longitudinal axis at a right angle to the source of light. It is best to have instrument and light rigidly fixed. Polariscopes mounted upon trestle supports are preferable to those upon tripods since a slight knock may swing the tripod out of alignment and cause a change in the zero point.

Variations in the brightness of illumination are also undesirable, and for accurate work the emission of light should be constant. The optical center of gravity of purified sodium light, for example, is 589.22 mμ for a certain average brightness of flame; variations in this brightness, however, may change the wavelength by 0.11 mμ with corresponding differences in the rotation of polarized light (25″ for a rotation angle of 20°). With salts of the alkalies and alkaline earths, increasing the brightness of flame (increase of vaporized salt per unit volume of flame) produces an irregular broadening of the spectral lines with a shifting of the mean wavelength toward the red end of the spectrum.

Gas Lamps for Sodium Light. Of the various polariscope lamps for sodium light only a few of the more common forms will be described. The lamp shown in Fig. 134 illustrates the essential principles of the usual gas sodium lamps. This consists of a Bunsen burner with side entrance for gas to prevent stoppage of the inlet through dropping of fused salt; the burner is surmounted by a chimney which can be adjusted to the desired height by the screw h. The holder for the fused salt consists of a grooved ring of porcelain or platinum, attached

to an upright support and can be moved in and out of the flame through a slot in the chimney by means of the screw *p*. The flame is adjusted so as to be colorless, with as strong an air blast as possible, so that the light may be free from incandescent carbon particles.

The hot part of the flame impinges upon the ring and produces a cone of sodium light. The fused salt must be renewed as fast as vaporized; a convenient means of effecting this renewal is shown in Přibram's[1]

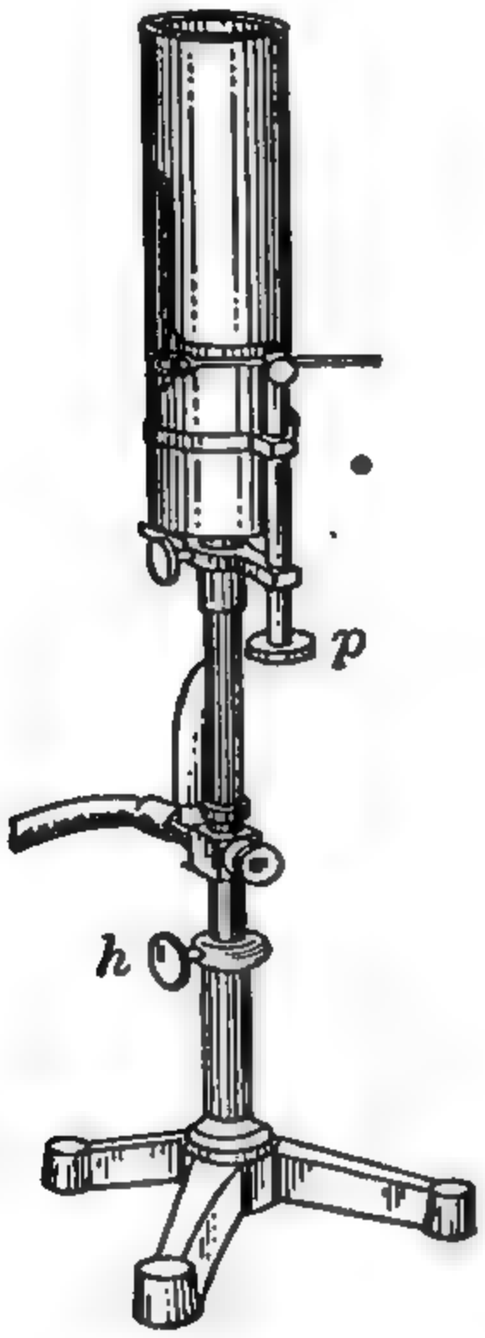


FIG. 134. Simple form of gas sodium lamp.

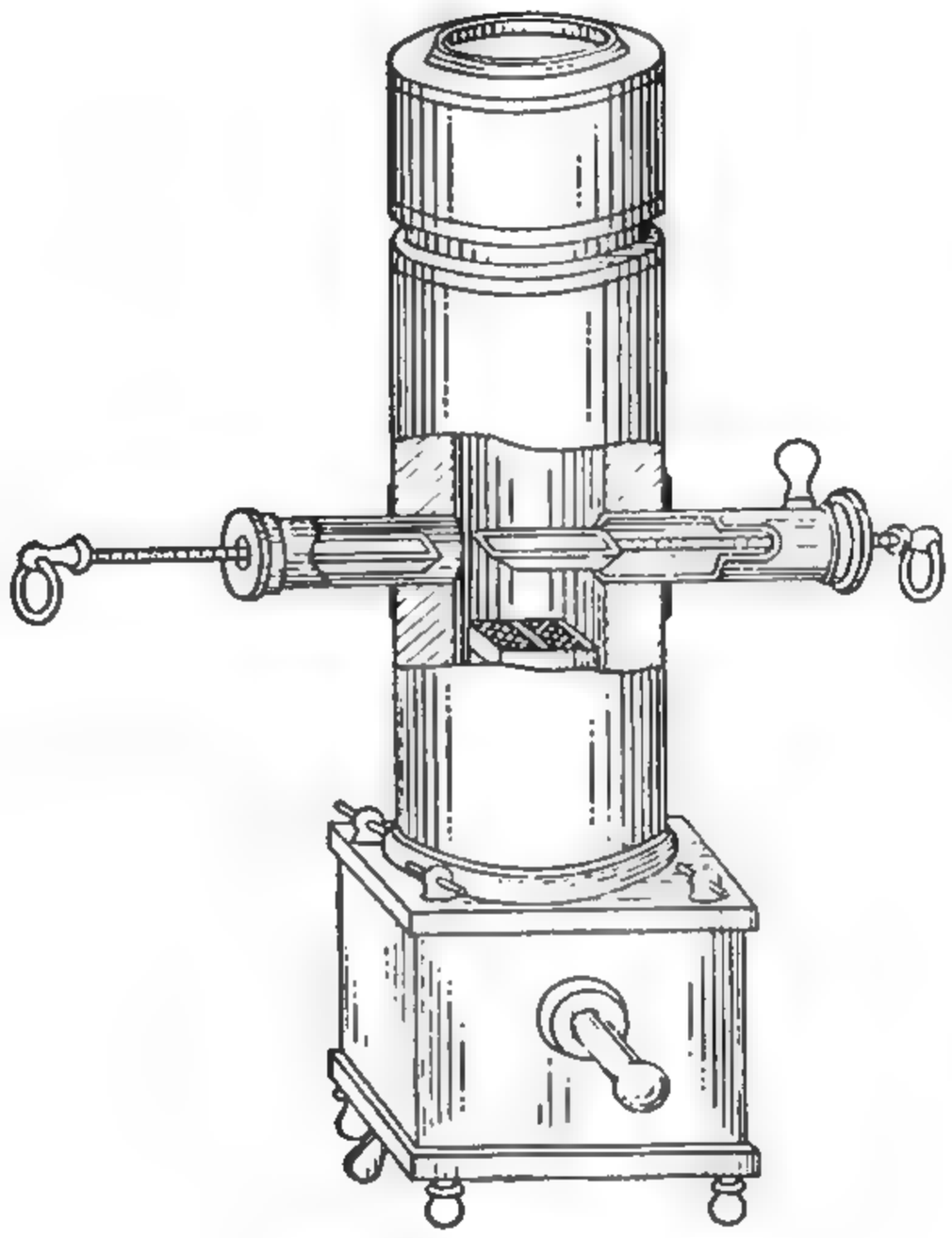


FIG. 135. Přibram's gas sodium lamp.

sodium lamp, Fig. 135, which contains two boats; the empty one is drawn out for refilling and the one in reserve inserted in its place.

The sodium lamp of Landolt[2] gives a more intense flame than either of the lamps just described. It consists of a powerful Muencke gas burner with a cylindrical chimney. Upon the latter are placed two heavy nickel wires supporting rolls of fine nickel wire netting which contains fused salt. The burner is surmounted by a second rectangular chimney of sheet iron with a movable brass door containing apertures of 20-, 15-, and 10-mm. diameter.

The simplest and cleanest of gas sodium lamps and the one giving the most continuous flame is that of Zeiss, Fig. 136. This is composed of

[1] *Z. anal. Chem.*, 34, 166 (1895).
[2] *Z. Instrumentenk.*, 4, 390 (1884).

an upper part *A*, capping an ordinary Bunsen burner and secured to it by means of a screw. The casting *A* carries the diaphragm screen *K*, out of which the rectangular opening *L* is cut, also the flat burner *C* producing a square flame, and a small support for the salt carrier *E*, which consists of a piece of pumice stone, measuring about 4 by 1 by ½ cm., saturated with salt. It is held upon the support by the spring clip *F* and can be regulated to the flame by means of the screw *J* operating on the spring *GH*. It is best to adjust the pumice stone so that it merely touches and tinges the flame. If *E* is inserted in the flame too deeply the flame is overcooled and a dark, rather sharply defined zone is produced. The flickering margins of the flame are cut off by the diaphragm *K*. A few minutes are needed for heating the pumice before the flame attains its maximum brilliancy, after which it will remain constant for hours together. The tablets of pumice stone saturated with salt are supplied by the trade at small cost.

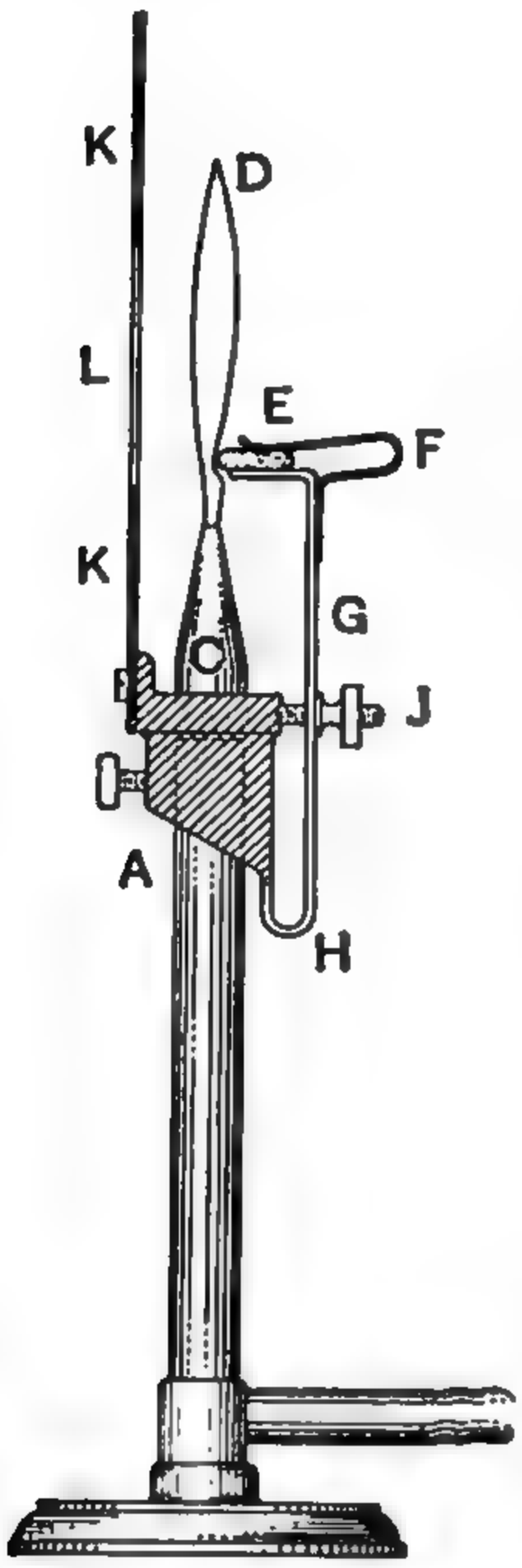
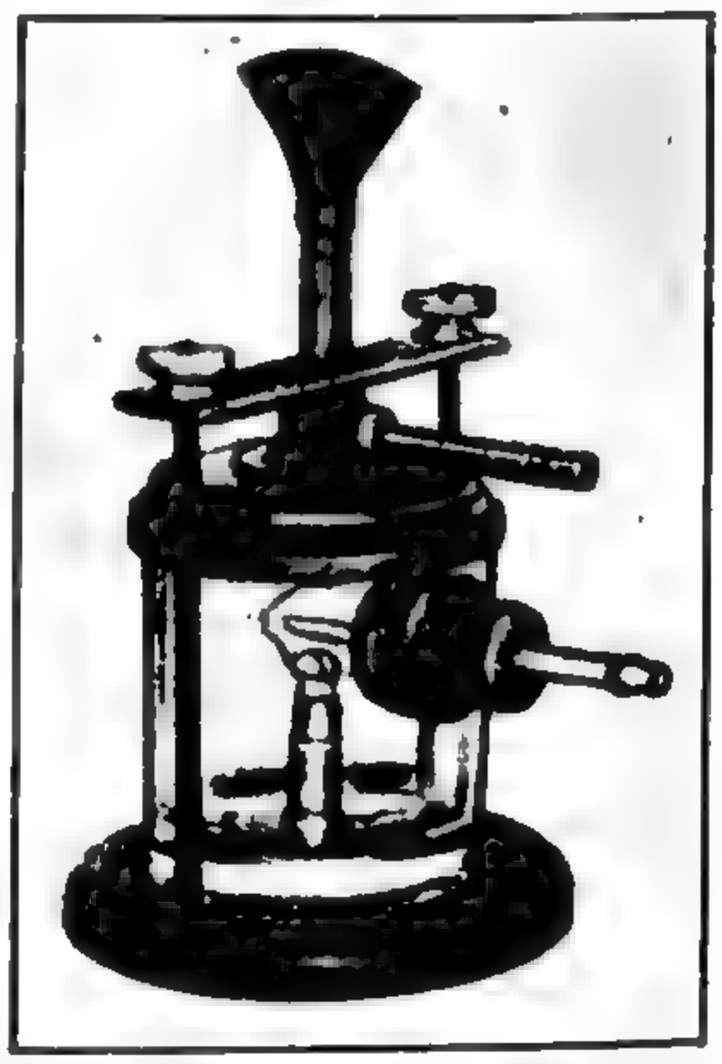


FIG. 136. Zeiss gas sodium lamp.

Another convenient form of sodium light which requires practically no attention is illustrated in Fig. 137.[3] It consists of a Bunsen burner in which the air supply, furnished by a small compressor, is first used to atomize a solution of sodium chloride. The spray is carried up with the air into the gas and imparts to it the characteristic color. In similar devices[4] the sodium salt is vaporized in a separate container by heat, and the vapor is introduced into the flame by the air supply of the burner. Various arrangements[5] in which the sodium chloride solution is conveyed to a Bunsen flame by means of an asbestos wick have also been described.



(Courtesy of Gaertner Scientific Corp.)

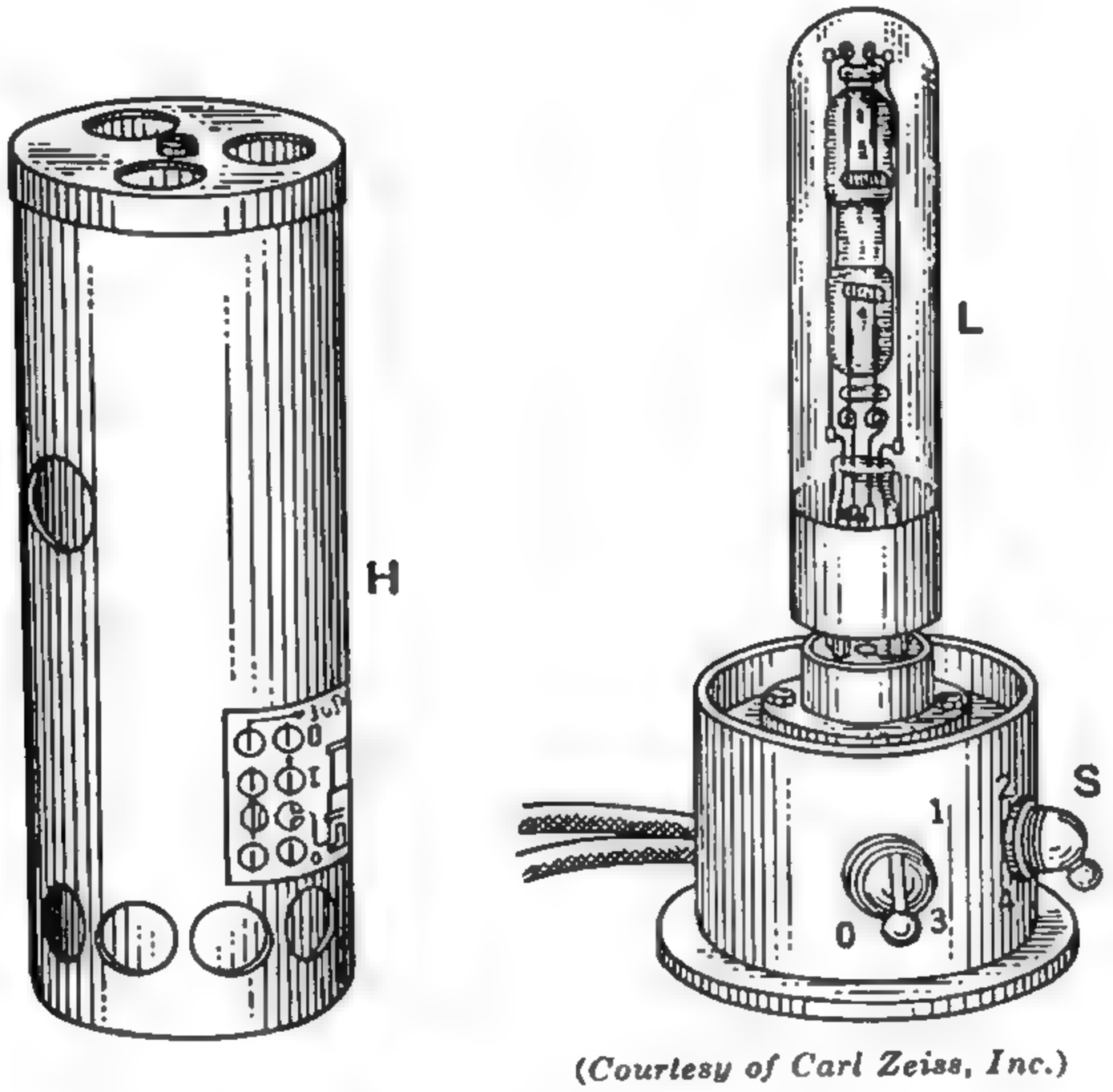
FIG. 137. Gaertner gas sodium lamp.

[3] Manufactured by Gaertner Scientific Corp., Chicago.

[4] Jones, *J. Soc. Chem. Ind.*, **42**, 459T (1923); Gordon, *J. Am. Chem. Soc.*, **47**, 1045 (1925).

[5] Manley, *Phil. Mag.*, **45**, 336 (1923); Dolid, *Ind. Eng. Chem.*, **16**, 53 (1924).

In place of common salt, sodium bromide is sometimes used for illumination. This gives a much stronger flame, but the vaporization is much more rapid than with salt and there is the additional disadvantage of the evolution of bromine vapors which may attack the instrument unless the lamp is placed under a hood. Pencils to be inserted into the Bunsen flame may, according to McLachlan and Middleton,[6] be made by mixing 33 parts sodium chloride, 33 parts sodium bromide,



(Courtesy of Carl Zeiss, Inc.)

FIG. 138. Zeiss electric sodium-vapor lamp.

and 14 parts magnesium oxide with water to form a damp mass, kneading this into 20 parts of sodium metasilicate, forming the mixture into the shape and size of a lead pencil, and drying in an air oven.

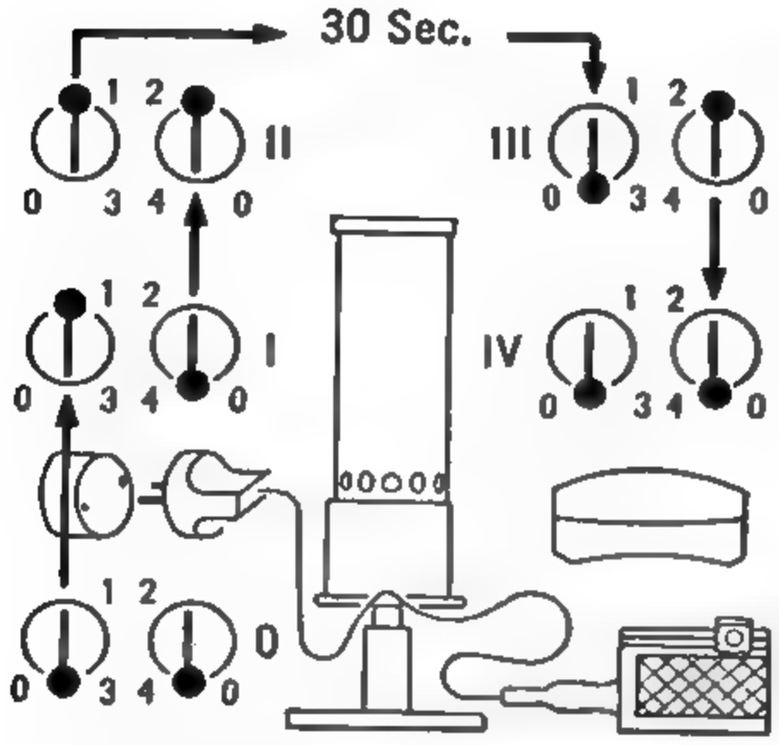
Sodium carbonate, sodium phosphate, sodium nitrite, and mixtures of these with salt in various proportions are also used for sodium lamps. Sticks of fused sodium carbonate heated in an oxygen blast lamp give a flame of great brilliancy, and this is the form of light recommended by Landolt[7] when intense illumination is desired.

Electric Sodium-Vapor Lamps. All gas sodium burners are inconvenient to handle, have a very hot flame, and require much attention. Most of them give light of low intensity, and it is always difficult to avoid fluctuations in the intensity and consequent shifts in the wavelength, as pointed out on p. 230. These disadvantages have

[6] *Analyst*, 52, 639 (1927).

[7] "Das optische Drehungsvermögen," 2nd ed., p. 359, 1898.

been overcome by the introduction of the electric sodium-vapor lamp. Various forms of this lamp, differing somewhat in appearance and manipulation but all based on the same principle, are on the market. The Zeiss sodium-vapor lamp, Fig. 138, is made for alternating or direct current, 110 or 220 volts. It consists of the lamp proper, *L*, provided with a 4-pole cap; a stand *S* with a socket for holding the lamp; and the housing *H*. The wiring and manipulation are explained in Fig. 139. Before starting the lamp, the two switches, marked 0,1,3 and 0,2,4, must be in the 0 (down) position. The socket at the end of one of the two cables is connected with the resistance box furnished with the lamp, and the two-prong plug at the end of the other cable is inserted into a line socket, after the polarity has been checked if direct current is used. The left-hand switch (0,1,3) is then turned to position I, and the right-hand switch (0,2,4) to position II. After about 30 seconds, the left-hand switch is turned back to position III, which lights the lamp. Full intensity is reached in about 2 minutes. To turn off the lamp, the right-hand switch is turned back to position IV.



(*Courtesy of Carl Zeiss, Inc.*)

FIG. 139. Switch diagram for Zeiss electric sodium lamp.

The electric sodium-vapor lamp is safer than the gas lamp, since there is no exposed flame and it creates very little heat. The light intensity is very much higher, and is quite constant after the maximum has been reached. This makes it possible to use the polarimeter where previously the more costly saccharimeter was required. It is only necessary to equip the polarimeter with a second scale, reading in per cent sucrose, for the particular normal weight to be employed. For the Herzfeld-Schönrock scale the 100° point lies at 34.650° circular, and for the Bates-Jackson scale at 34.613°.[8] While in the usual saccharimeters, excepting the Jobin and Yvon and the Bates instruments, the half-shadow angle is fixed, it can be adjusted in the polarimeter, either widened to read dark-colored solutions or narrowed to increase the accuracy of the reading. Exact temperature control is also much easier, because the instrument itself need not be adjusted to the standard temperature, but only the solution, whereas with the saccharimeter the quartz wedges must also be at the standard temperature. It must also be considered that perfectly homogeneous

[8] Landt, *Intern. Sugar J.*, 37, 474 (1935). The difference between these and the generally accepted values is only 0.02° S. and may usually be neglected.

quartz for the wedges is difficult to obtain and that for this reason intermediate values on the saccharimeter scale are likely to be incorrect even though the 0 and 100 points are correctly set. Polarimeters equipped with an electric sodium lamp and a sugar scale have been described in Chapter VI.

According to Pucherna[9] the electric sodium lamp is to be preferred even for illuminating quartz-wedge saccharimeters, because there are no differences in the tint of the two halves of the field, and greater accuracy of reading is attained.

Purification of Sodium Light. For accurate polariscope measurements it is necessary to purify the sodium light from other rays. This can be done either by use of light filters or by spectral separation of the extraneous rays.

Sodium light can be freed from most of the foreign rays at the violet end of the spectrum by means of bichromate solution, which has a strong absorption band in the green and blue. The rays at the other end of the spectrum can be removed by uranous sulfate solution, which has a strong absorption band in the red. A combination of these two solutions, as in the Lippich light filter, constitutes the most effective absorbent means of sodium-light purification known.

Lippich Light Filter. The Lippich light filter consists of a tubular cell closed at the ends by tightly fitting cover glasses and divided by a glass plate into two smaller cells of unequal size. The larger cell, 10 cm. long, is filled with a 6 per cent filtered solution of potassium bichromate; the smaller cell is filled with a solution of uranous sulfate, $U(SO_4)_2$, prepared as follows: 5 g. of purest uranyl sulfate, $UO_2SO_4 + 3H_2O$, is dissolved in 100 ml. of water, and 2 g. of powdered chemically pure zinc is added; 3 ml. of concentrated sulfuric acid is then added in 1-ml. portions, waiting each time until the evolution of hydrogen has nearly ceased; the flask is corked during the reaction, and is allowed to stand about 6 hours, when the solution is filtered and the cell immediately filled in such a way as to leave only the smallest possible bubble of air behind. After standing for a day the cell is ready for use; the uranous solution retains its stability for 1 to 2 months, or until its deep green color is changed by oxidation into the yellow of the uranyl compound, when the cell must be refilled with fresh solution. The weights and volumes prescribed for making up the absorbent solutions must be rigidly adhered to.

Landolt gives the following average wavelengths for sodium light from different sources in which the wavelength of the D_1 line is placed at 589.62 mμ and the D_2 line at 589.02 mμ.

[9] *Z. Zuckerind. čechoslovak. Rep.*, **60**, 102 (1935/36).

TABLE XXXVII

Wavelength of Different Kinds of Sodium Light

No.	Source of Light	Purification	Wavelength in mμ
1	Bunsen flame with NaBr	10-cm. layer of 9 per cent $K_2Cr_2O_7$ in water	592.04
2	Bunsen flame with NaCl	10-cm. layer of 9 per cent $K_2Cr_2O_7$ in water	589.48
3	Burner with NaCl or NaBr	Lippich filter $K_2Cr_2O_7$ and $U(SO_4)_2$	589.32
4	Sodium light	Perfectly spectral pure; light of only the two D lines	589.25
5	Landolt lamp with NaCl	1.5-cm. layer of 6 per cent $K_2Cr_2O_7$ in water	588.94
6	Bunsen flame with NaCl	10-cm. layer of 9 per cent $K_2Cr_2O_7$ in water and 1-cm. layer of 13.6 per cent $CuCl_2$ in water	588.91
7	Landolt lamp with NaCl	Unpurified	588.06

Similar figures have been obtained by Schönrock.

The Lippich light filter gives a wavelength exactly between the two D lines of sodium and agreeing very closely with that obtained by spectral purification. In all cases where light filters are used the solutions must be placed between lamp and condensing lens (*B*, Fig. 133).

The selective filters mentioned above are to be used only in connection with a sodium burner. Filters have also been described for the purpose of obtaining from white light a narrow spectral band whose effective wavelength is close to that of the D line. Landolt advocated a Welsbach gas lamp the light of which was passed successively through solutions of nickel sulfate, potassium chromate, and potassium permanganate.[10] The intensity of the light is so much reduced by absorption in the filters that the half-shadow angle must be set at not less than 8°. Schoorl[11] has used a 50-candlepower electric light, and a 2-cm. layer of a solution containing 4.4 g. crystallized copper sulfate and 4.7 g. potassium dichromate in 100 ml. These devices, however, are not to be recommended for polarimetric work in general, because the light they furnish is not of sufficient purity.

Spectral Purification of Sodium Light. This system of purification, employing dispersion by prisms, is the most thorough of all methods of purification. It is required, however, only for measurements of

[10] " Das optische Drehungsvermögen," 2nd ed., p. 388, 1898.

[11] *Pharm. Weekblad,* **63**, 21 (1925); *Chem. Weekblad,* **23**, 113 (1926).

the highest precision. A convenient device for the purpose is the Schmidt and Haensch monochromator shown in Fig. 105. It consists essentially of a direct-vision spectroscope, with a dispersion of 5.5°. The light enters through slit Sp_1. The micrometer screw M is used to set the collimator to any desired wavelength, e.g., the D line. The light then passes through the prisms and enters the polarimeter through slit Sp. The lamp, connected with the line current through a rheostat, illuminates the measuring circle of the polarimeter through a system of mirrors. The entire monochromator is mounted on a heavy stand which can be fastened to the trestle stand of the polarimeter.

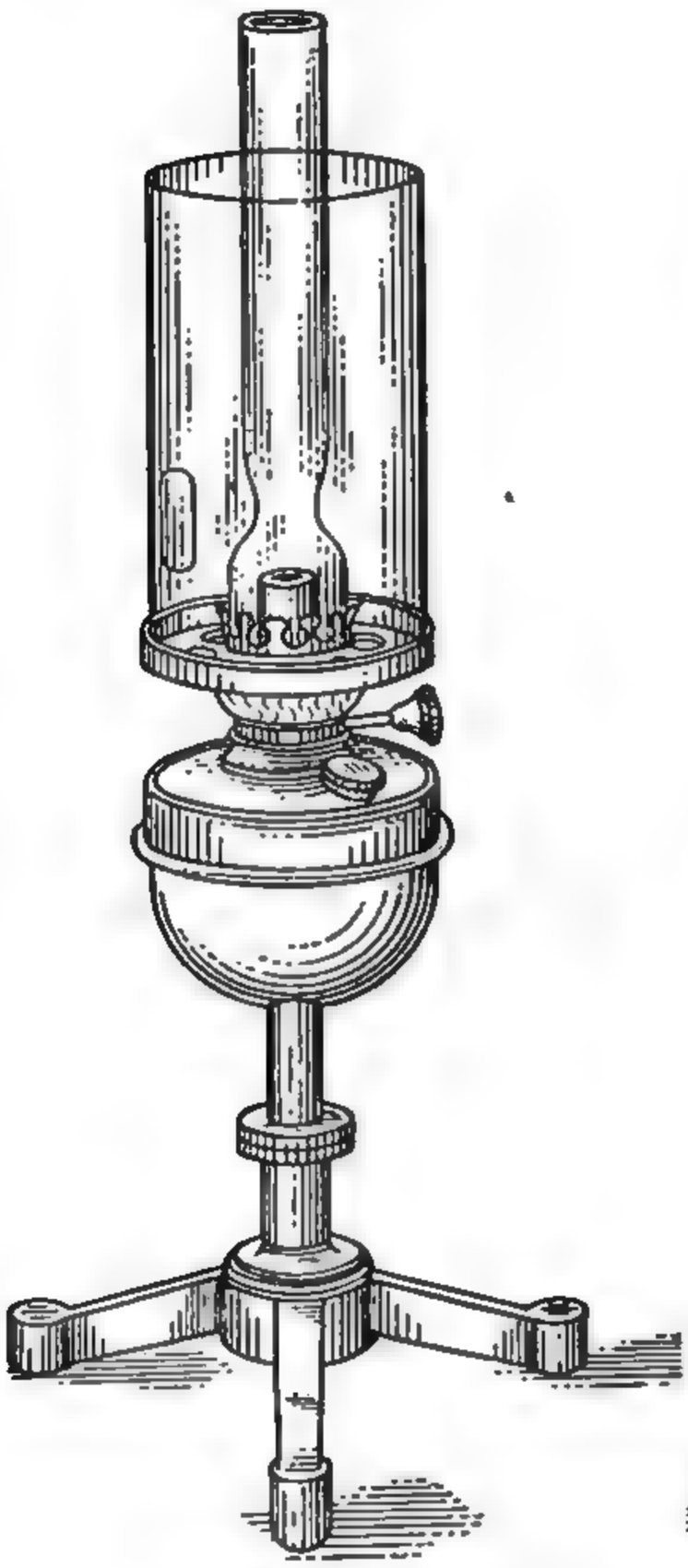
Other Monochromatic Light Sources. Although sodium light has been used almost exclusively for polarimetric investigations because of the ease with which it may be produced, and although the difficulties experienced with gas burners have been overcome by the electric sodium-vapor lamp, other objections have been raised against it. The principal line is a doublet whose components are 0.6 $m\mu$ apart, and there are other fairly intense lines in the neighborhood of the D lines which are not easily excluded even by spectral purification. The National Bureau of Standards[12] has therefore proposed the green mercury line at 546.1 $m\mu$ for fundamental investigations of specific rotation. The extreme distance between the accompanying spectral lines is only 0.04 $m\mu$, and the spectral purity is therefore much higher than that of the D line of sodium. A number of mercury-vapor lamps are commercially available. The Zeiss electric vapor lamp, described previously, may be obtained equipped not only with sodium or mercury, but also with zinc, cadmium, thallium, potassium, rubidium, cesium, or neon. With all these light sources, spectral purification is effected, as with sodium light, by means of appropriate light filters or by means of the monochromator mentioned above, depending on the desired purity of the lines. These lamps are very convenient for the determination of rotatory dispersion.

Lamps for White Light. For illuminating polariscopes and saccharimeters with white light, a large number of lamps have been devised for use with oil, alcohol, gas, acetylene, and electricity.

A convenient form of oil lamp with single burner and adjustable support is shown in Fig. 140, and a similar one, with duplex burner, in Fig. 141. Both are provided with asbestos chimneys, with an opening for the light to pass through. In some oil lamps a focusing lens is placed in the aperture of the chimney; this should be removed as it may cause an incorrect passage of the beam of light through the polariscope.

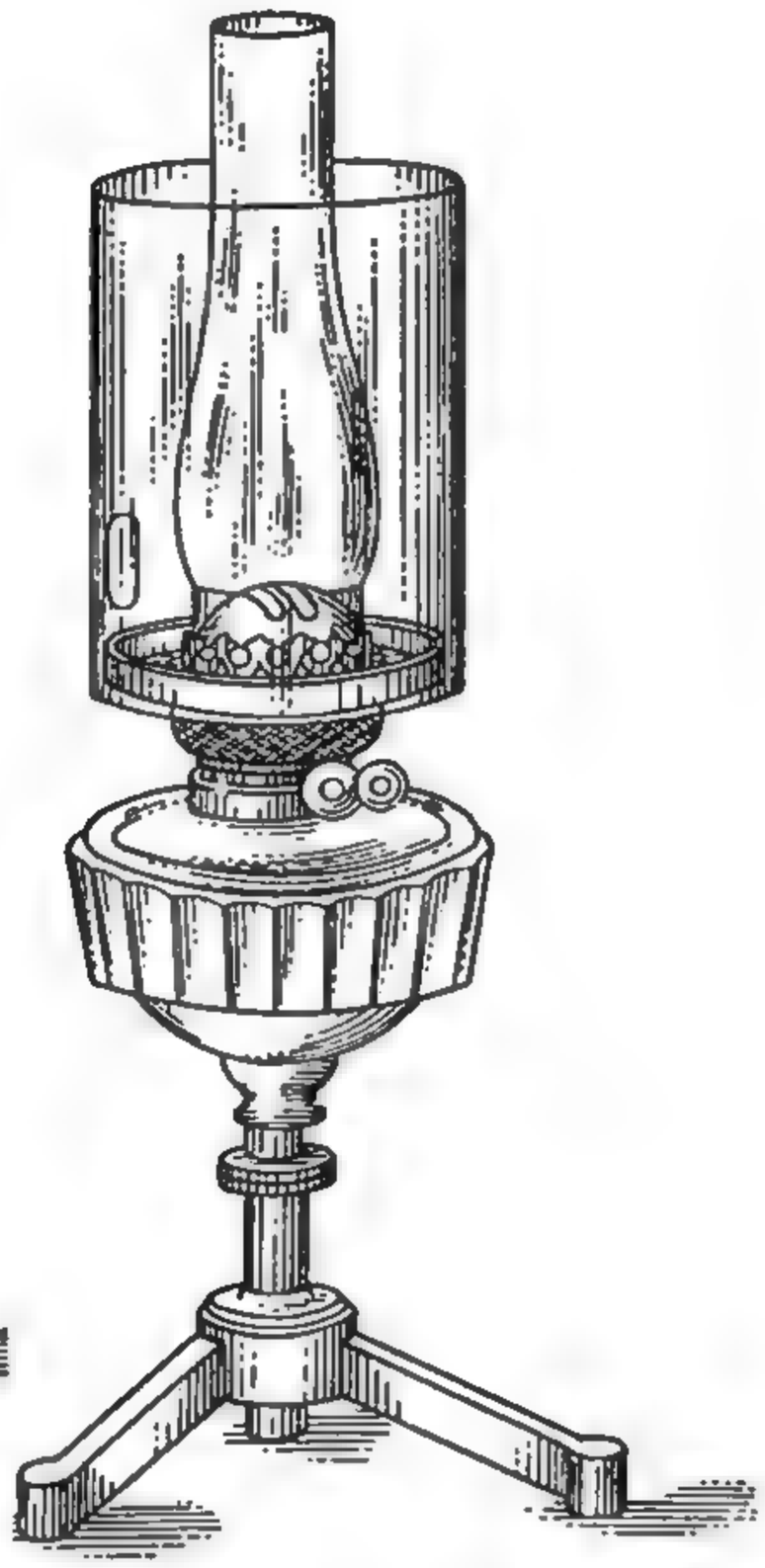
[12] *Bur. Standards Circ.* **44**, pp. 14–17, 1918.

The best forms of gas lamp for illuminating are those provided with an Auer or Welsbach mantle (Fig. 142). The outer cylinder of these lamps, composed of sheet metal or asbestos, contains an opening whose lower part is covered with a plate of ground glass for diffusing the light; the upper uncovered part of the opening serves for illuminating the polariscope scale. A form of lamp for burning alcohol somewhat similar in design to the above is shown in Fig. 143. Gas burners for



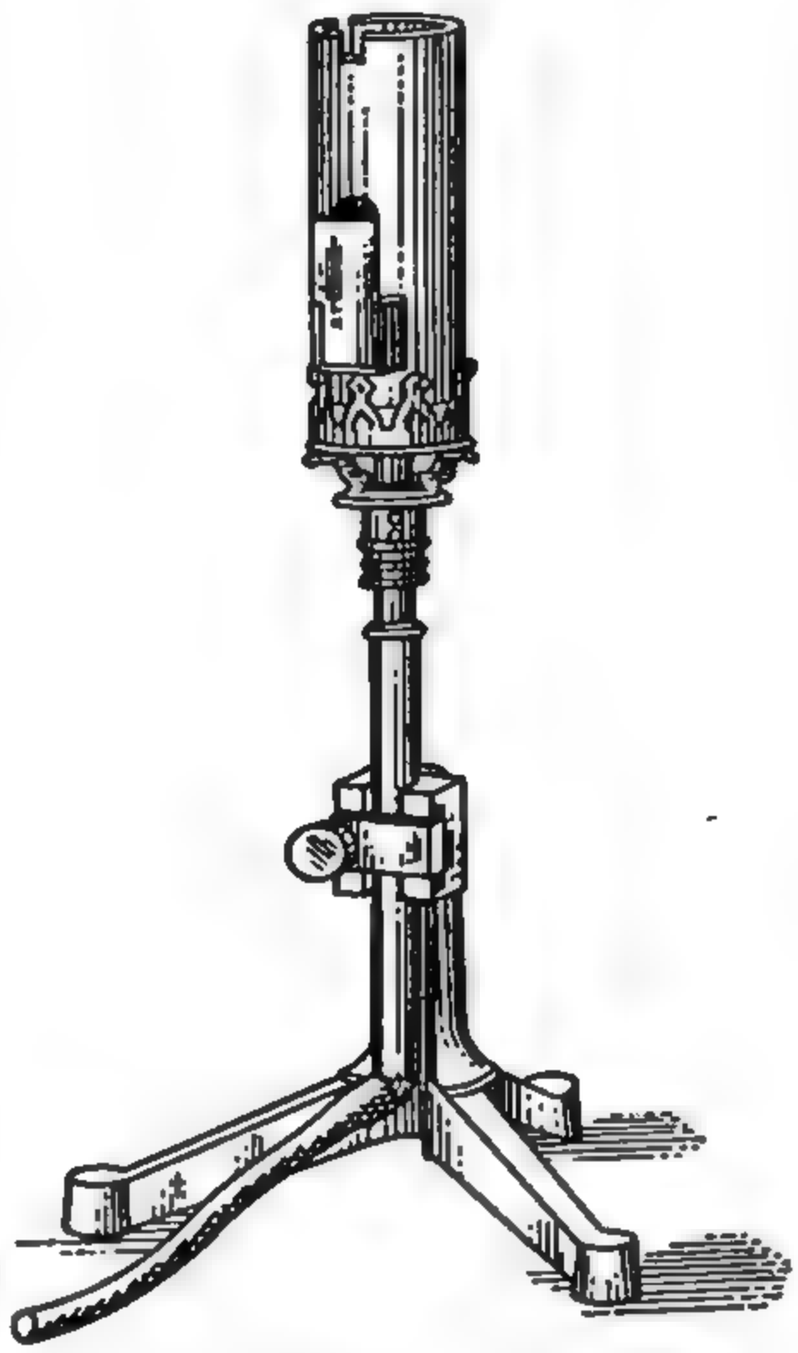
(*Courtesy of Akalos, Inc.*)

FIG. 140. Oil lamp with single burner.



(*Courtesy of Akalos, Inc.*)

FIG. 141. Oil lamp with duplex burner.



(*Courtesy of Akalos, Inc.*)

FIG. 142. Gas lamp with Welsbach mantle.

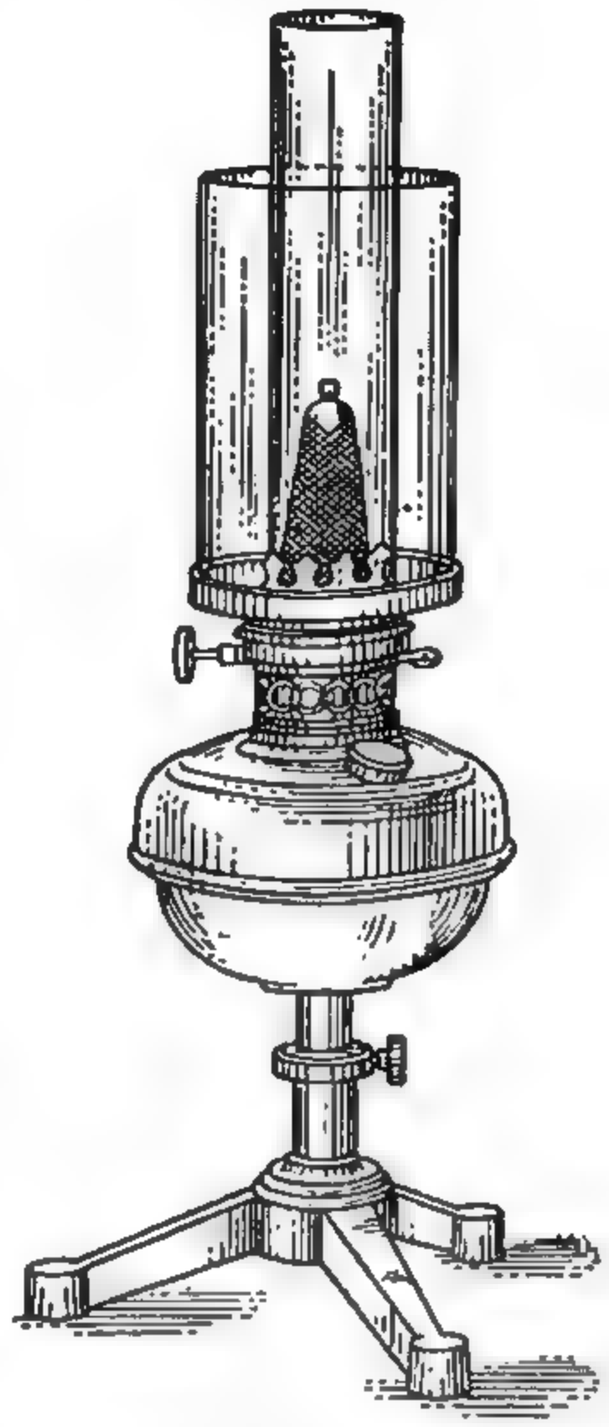
producing lime or zircon light are also used for illuminating polariscopes. Acetylene lamps of 25 to 50 candlepower give a light of great brilliancy and are especially valuable upon sugar plantations where gas or electricity is not available. The acetylene lamps should be fitted with cylinders similar to those in Figs. 140 or 142.

For electrical illumination incandescent lamps with a concentrated filament are the best, furnishing great intensity of illumination. A 100-watt "Spotlight" Mazda lamp (Fig. 144) is very suitable; similar

lamps with a cylindrical instead of spherical bulb are available. The Pointolite lamp is also recommended. All these lamps should be mounted in asbestos or metal cylinders similar to that in Fig. 142; a plate of ground glass is necessary for diffusing the light, otherwise the irregularities in source of emission will not be sufficiently equalized for obtaining a uniform field.

A saccharimeter lamp with bichromate cell devised at the National Bureau of Standards, is shown in Fig. 128, p. 220.

A small electric attachment constructed by Schmidt and Haensch for illuminating their saccharimeters is illustrated in Figs. 122 and 145. The small osram lamp, shown near the current outlet, Fig. 122, is adapted for a 6-volt current which is supplied from the main line, being connected in series with the lamp shown near the front end of the saccharimeter. This lamp is also used to illuminate the notebook of the observer. The apparatus itself is screwed on the polarizing end of the saccharimeter after removal of the rear tube from the saccharimeter. The light from the 6-volt lamp passes through a condensing lens. As the horizontal filament of the lamp is not always quite concentric to the frame, the necessary adjustment can be made by means of screw *J* which acts on the condensing lens. The mirror Sp_1 reflects part of the light, to illuminate the quartz-wedge scale, as shown in Fig. 121.



(*Courtesy of Akatos, Inc.*)

FIG. 143. Alcohol lamp with Welsbach mantle.

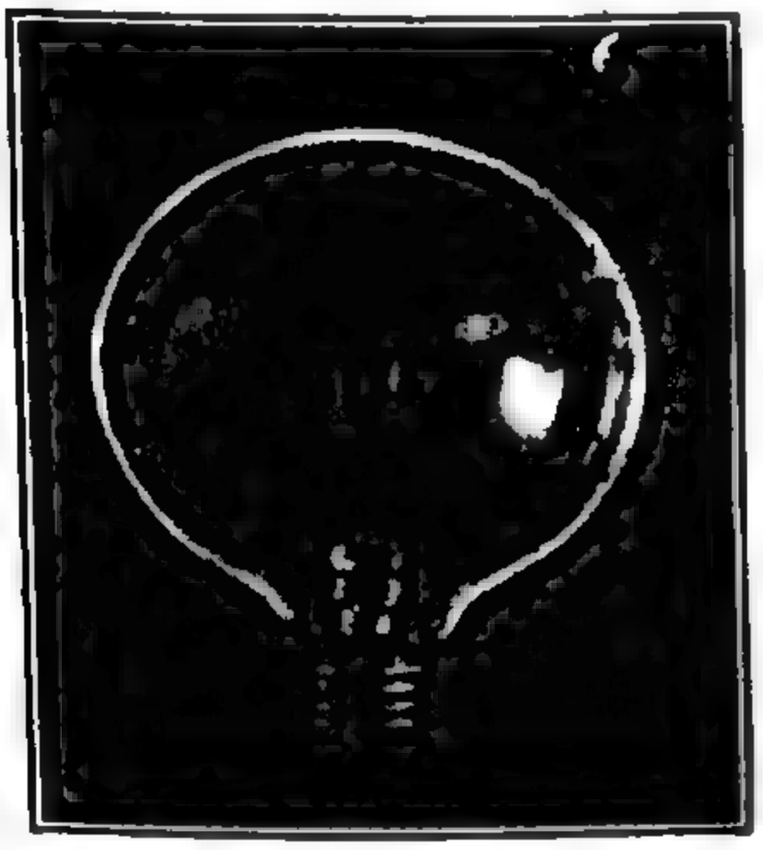
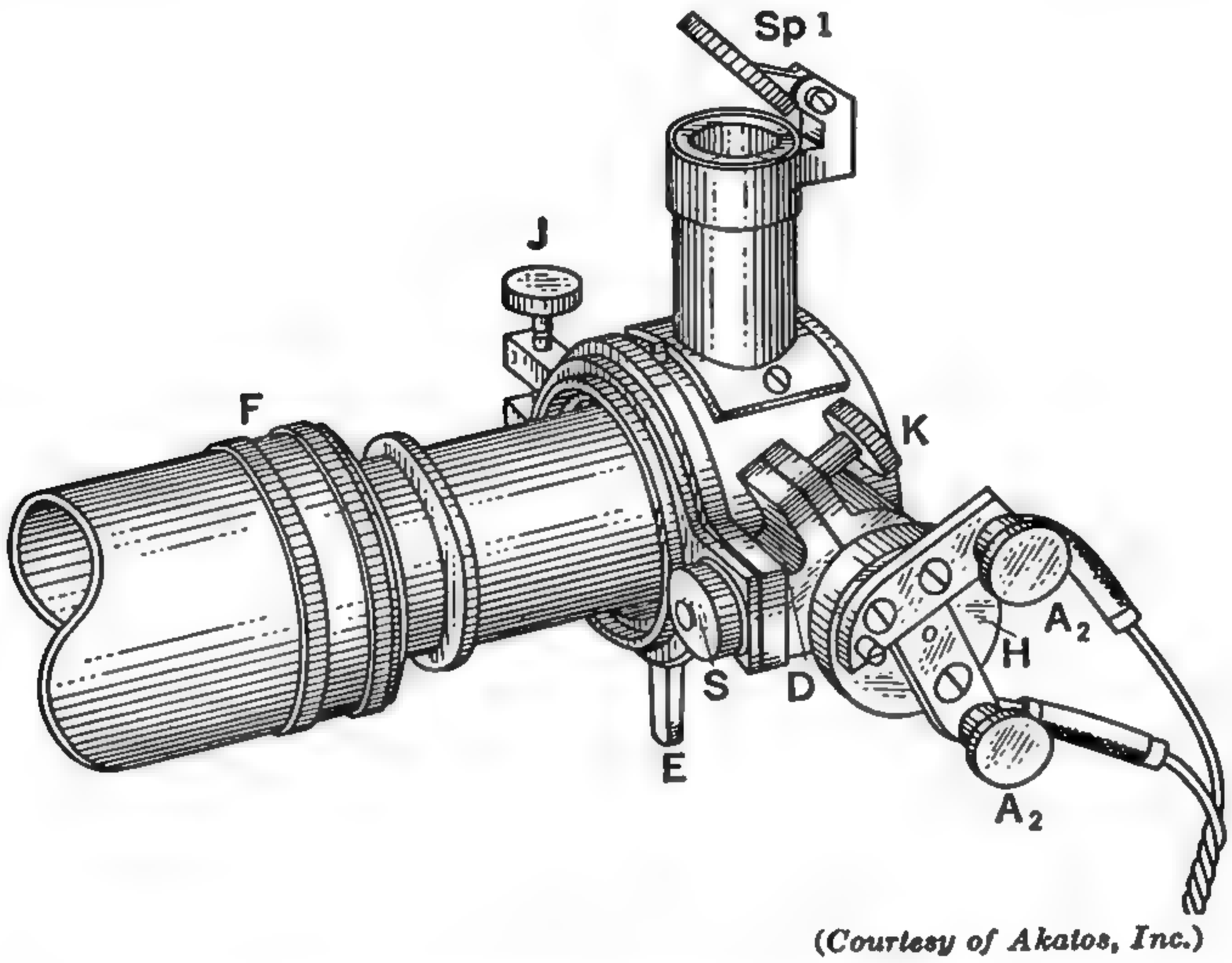


FIG. 144. Spotlight electric lamp.

POLARISCOPE TUBES

For retaining sugar solutions during polarization there are a variety of tubes of different construction, form, and length. In the selection of these the chemist must be guided more or less by the nature of his work. All tubes, however, when accuracy of observation is desired, must conform to three general requirements: (1) the length of the tube must be accurately fixed; (2) the ends of the tube and the surfaces of its cover glasses must be plane parallel; (3) the tube

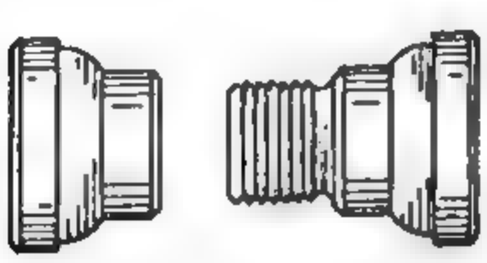
must be centered evenly in its mountings and, when fitted with its caps, should be free from eccentricity. There are other minor requirements of tube construction which will be given under the description of the different forms.



(Courtesy of Akatos, Inc.)

FIG. 145. Schmidt and Haensch electric illuminating attachment.

Figure 146 shows the most common and simplest forms of glass polarization tubes. These and other forms of tube are usually supplied in lengths of 25, 50, 100, 110, 200, 220, 400, 500, and 600 mm.; for special kinds of work tubes several meters long have been constructed.



25-mm tube

100-mm tube

200-mm tube

FIG. 146. Forms of plain glass polariscope tubes.

A tube of 200-mm. length is used for the normal weight of all saccharimeters. If, on account of depth of color, a 100- or 50-mm. tube is employed and the resultant reading is recalculated by multi-

plying by 2 or 4, there is, of course, a corresponding doubling or quadrupling of the errors of observation; short observation tubes are to be used therefore only in extreme cases. With very dilute sugar solutions and with sugars or sugar mixtures of low specific rotation the 400- or 600-mm. tube will increase the accuracy of the observation, provided that the color is not too great to disturb the reading. Tubes of odd lengths, such as 55, 110, and 220 mm., should be distinctly marked lest they be confused with the 50-, 100-, and 200-mm. sizes.

Mounting of Polariscope Tubes. The ends of the glass observation tubes are cemented into metal mounts which are threaded for the purpose of receiving the screw cap. Litharge and glycerin make a much better cement than the waxy material employed by some manufacturers. The wax, especially on warm days, softens readily and when in this condition there is danger in screwing on the cap of drawing the mount from its setting so that it projects slightly beyond the ends of the tube; the length of the column of liquid to be polarized may thus be increased and a considerable plus error introduced in the observation. The ends of the glass tubes should project only slightly beyond the threaded heads; if too much of the end is exposed there is danger of chipping or breakage. The chemist should not attempt to reset his tubes unless he has a small lathe in which they can be centered and revolved while the cement is hardening; otherwise the tubes may not be evenly mounted.

A simple means of testing for eccentricity of mounting is to place the tube, with caps screwed on, in the trough of a polariscope and while giving it a rotatory motion to view the opening through the tube with reference to the polariscope field. If the tube has been properly centered and the caps are free from eccentricity the tube opening will remain in the center of the field and show no wabbling movement during rotation. To test for plane parallelism of the ends of the tube and of cover glasses, the experiment just described is repeated with the cover glasses in position and the tube filled with water. If the ends of the tube have not been ground squarely across or the cover glasses are not plane parallel, the opening of the tube will wabble perceptibly during rotation owing to the refraction of light through the water from the inclined surfaces of the cover glasses. A difference of several tenths of a Ventzke degree may be noted between the readings of a tube in different positions through lack of plane parallelism in ends or cover glasses. According to Landolt the angle between the opposite ground-end surfaces of a polariscope tube should always be less than 10′ and the angle between the two planes of a cover glass less than 5′. The small angles of inclination between planes of cover

glasses and between ends of tubes not exceeding 200 mm. in length are measured by a spectrometer.

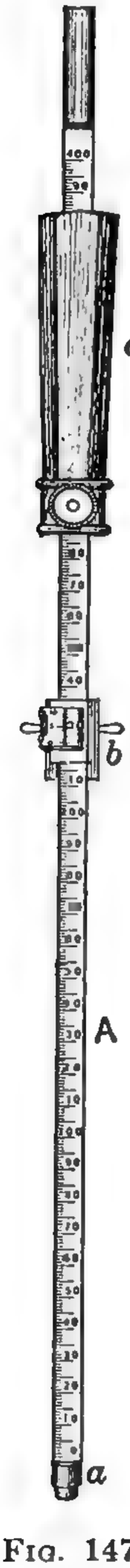


Fig. 147. Landolt's gauge for calibrating polariscope tubes.

Calibration of Polariscope Tubes. A most convenient means of calibrating the length of polariscope tubes is the measuring gauge of Landolt, shown in Fig. 147. This gauge, which has an adjustable handle *c*, consists of a measuring rod *A* of steel graduated for a distance of 400 mm. and provided with a sliding vernier *b* which gives readings to 0.1 mm. The lower end of the rod and the bottom of the vernier are provided with knife edges. When the knife edge of the rod is placed upon a smooth hard surface, such as glass, and the vernier brought down until its knife edges are in close contact with the same surface, the 0 points of scale and vernier should agree. If there is lack of agreement, the 0 point of the vernier may be either adjusted or the difference noted and applied to all readings. To calibrate an observation tube, one end of the tube is closed with its cover glass and cap, and after the tube is placed in an upright position with the closed end down the measuring rod is inserted until its knife edge touches the cover glass; the rod being held perfectly upright, the vernier is slipped down until its knife edges coincide with the upper end of the tube; the reading of the scale and vernier will then give the length of tube. Other readings are made, rotating the rod a little each time from its original position, and the average is taken. Calibration of tubes should be made at the standard temperature 20° C.; if measurements are made at temperatures very different from this the changes in length of tube and gauge due to expansion or contraction must be taken into account (coefficient of expansion in length 1° C. for steel = 0.000013 and for glass = 0.000008). Measuring gauges can be verified as to accuracy at the National Bureau of Standards.

The measuring gauge of Landolt will detect an error of 0.1 mm., which is equivalent to an error of 0.05° V. for a sugar solution polarizing 100° V. in a 200-mm. tube. This is sufficiently close for ordinary saccharimetric measurements; if a finer determination of tube length is desired the measurement must be made upon a comparator; by means of this instrument measurements can be made to 0.01 mm.

Cover Glasses. The cover glasses used upon polariscope tubes must be of strong, colorless, and optically inactive glass; their surfaces must be plane parallel and free from cracks or scratches. In screwing the caps upon observation tubes, care must be taken that no severe pressure is brought to bear upon the cover glasses; otherwise the strain will render the glass optically active and produce serious errors in the observation. If a cover glass is optically active turning the tube in the trough of the polariscope will usually show variations in the intensity of the field with considerable difference in the reading for various positions of the tube. The practice of rotating the observation tube between readings is always a good one; in this way errors due to defective cover glasses, bad washers, pressure of caps, eccentricity, etc., may be detected which would otherwise escape notice. Cover glasses which have been rendered optically active through pressure should not be used for a day, in order that sufficient time may elapse for readjustment to neutrality.

Washers. Another common source of error in polariscopic work are badly fitting rubber washers in the screw caps of the tubes. The washers should be of soft rubber and lie evenly against the back of the cap without the slightest marginal elevation; otherwise the washer in tightening the cap may give the cover glass an inclined position and cause a considerable increase in the reading.

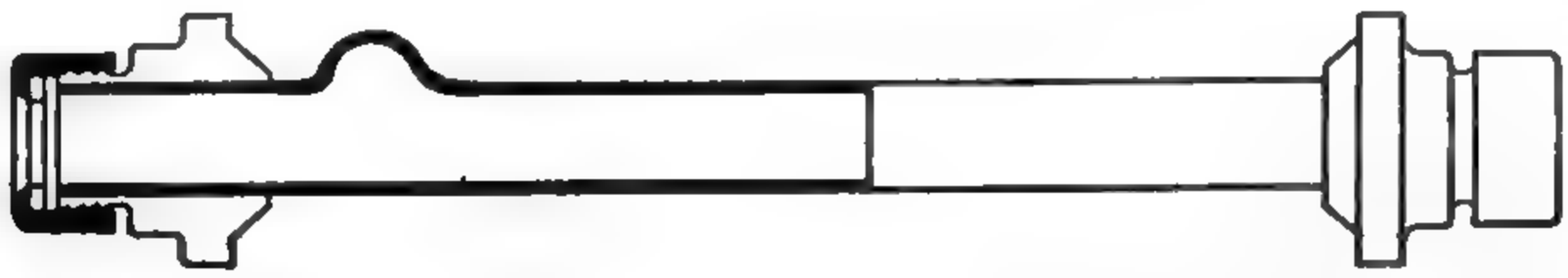
Special Forms of Polariscope Tubes

Tube with Enlarged End. Another form of glass polarization tube which presents several advantages is the tube with one end enlarged (Fig. 148). The enlargement serves as a receptacle for any air bubbles which may be enclosed with the liquid; the retention of a

Fig. 148. Schmidt and Haensch polariscope tube with enlarged end.
(Air bubbles are collected at point *a*, outside of the field of vision.)

small air bubble in the tube is in fact desirable since, by moving the bubble through the liquid from end to end before reading, slight differences in temperature are equalized, and no troublesome striations, due to currents of solution of different temperature, are present to distort the field. Instead of one end of the tube being enlarged, a

small bulb may be blown at any desired point along the length of the tube, as shown in a design by Bellingham and Stanley, Fig. 149.[13] The tube does not rest on the caps, but on special shoulders. It cannot be rotated, however, because the bulb must be in the upward position



(Courtesy of Bellingham and Stanley.)

FIG. 149. Bellingham and Stanley polariscope tube with bulb.

when the reading is taken. Tubes without enlargement must not retain air bubbles with the liquid; if striations are present the tube must remain at rest until the solution has reached equilibrium. The most frequent cause of a striated field is the warming of the solution in the tube by the hand; for this reason tubes should be handled only by the metal caps when being placed in the instrument.

Landolt's Tube. To prevent the liability of excessive pressure upon cover glasses, Landolt has devised a tube with sliding cap, which is pushed into position over the metal mount (Fig. 150*a*). The

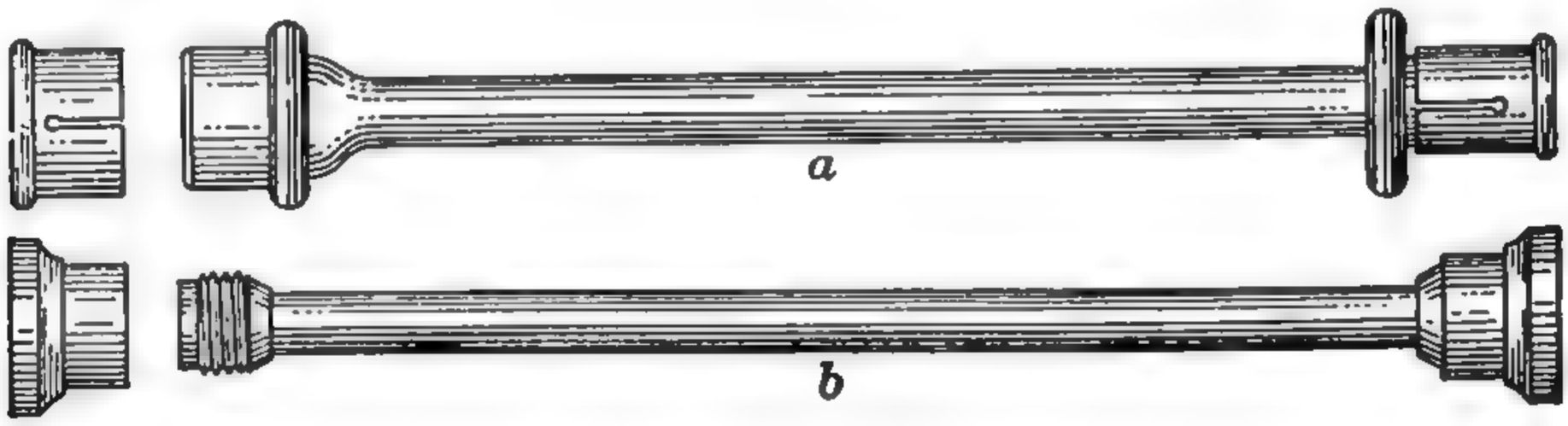


FIG. 150. (*a*) 200-mm. Landolt polariscope tube with enlarged end.
(*b*) 200-mm. metal polariscope tube.

French manufacturers also provide a cap that is shoved on and fastened with a bayonet catch.

Ninegar Tube. A bayonet cap with a spring which exerts enough pressure on the rubber washer to prevent leakage, but without causing optical activity in the cover glass, has been designed by Ninegar.[14] The tube itself is made of glass, protected by a surrounding metal armor which can be easily removed. The tube rests on collars and can be rotated in the trough without disturbing the caps. Tubes with screw

[13] Pellet, *Intern. Sugar J.*, **19, 76 (1917)**.

[14] Manufactured by Precision Scientific Co., Chicago.

caps are generally preferred and, if care is taken not to draw them up too tightly, will be found to answer all requirements. When observation tubes are used in large numbers it is a great advantage to have all caps interchangeable.

Metal Polarization Tubes. Polarization tubes of brass or nickel or other metal are preferred by many chemists. Such tubes, a form of which is shown in Fig. 150*b*, have the advantage of greater durability, but the disadvantage of being susceptible to the attack of acids (as in the method of inversion) or other corrosive liquids. Brass tubes have also more than twice the coefficient of expansion of glass tubes, the coefficient (β) for 1° C. being 0.000008 for glass and 0.000019 for brass. For glass and brass tubes measuring exactly 200 mm. at 20° C., the length at 35° C., $L_{t°} = L_{20°} [1 + \beta (t° - 20)] = 200.024$ mm. for glass and 200.057 mm. for brass, errors in length of no great significance. A more serious objection against metal tubes is the danger of their being bent out of alignment through hard or long usage. A knock or fall may cause a metal tube no apparent injury yet may bend it sufficiently to produce a considerable error in the polariscope reading. A number of brass polariscope tubes, submitted for examination, were so badly out of alignment that rotating the tubes in the trough of the polariscope caused a difference of more then 0.2° V. in the reading.

FIG. 151. Bates's polariscope tube.

Metal tubes have the further disadvantage that the solution cannot be seen in the tube except longitudinally, and that they cannot be marked easily for identification in the polarizing closet, whereas a serial number can be etched on glass tubes and seen by holding the tube against the light from the saccharimeter lamp. Metal tubes corrode with long usage, and they do not drain as well as glass tubes.

Most of the defects of metal tubes have been overcome in a design by Bates, Fig. 151. They are made with thicker walls than the old type and are therefore less liable to bend. The bore is 9 mm. and this makes it possible to utilize the full aperture of the polarizing system. Both ends are enlarged, which makes the cost of cover glasses and washers a little higher, but requires only one size of them. The

weight of the tube in the trough is carried on special shoulders, as in the tube of Fig. 150*a*. This prevents accidental loosening or tightening of the caps when the tube is rotated in the trough.

Pellet's Tube for Continuous Polarization. In the polarization of a large number of solutions in succession, as in the analysis of sugar beets, juices, etc., the Pellet tube for continuous polarizations is often of great use. Sections of this tube, which is made of metal, are shown in Fig. 152. Another form is that equipped with Ninegar bayonet caps (p. 244). The ends of the tube are closed and after the tube is placed in the instrument the solution to be polarized is

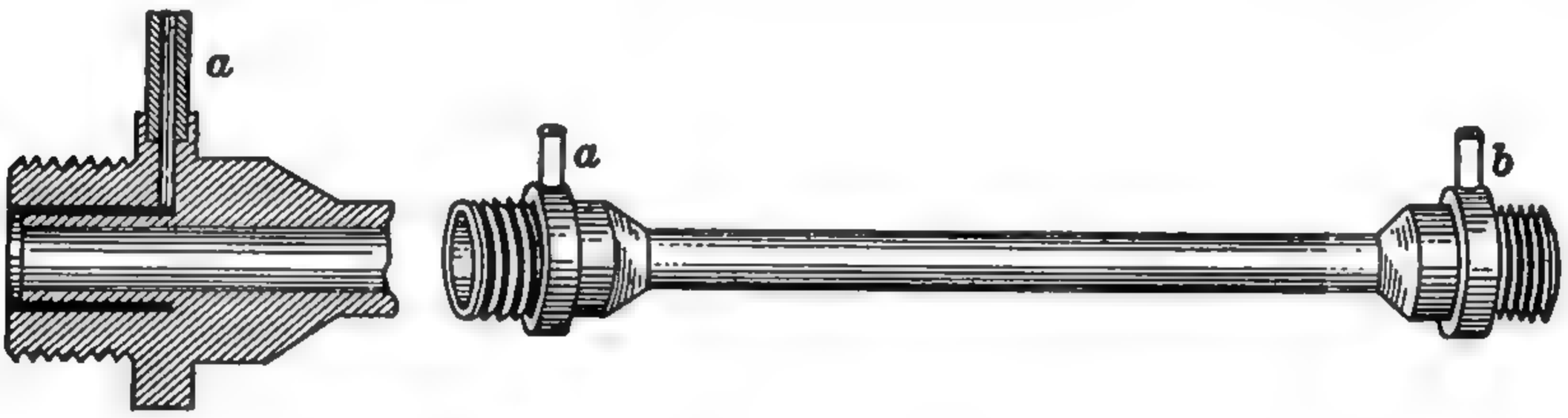


FIG. 152. Pellet's tube for continuous polarization.

poured through a small funnel into one of the nipples, *a* or *b*, the excess escaping through an exit tube connected by rubber tubing to the nipple at the opposite end. As soon as the solution is polarized, the succeeding solution is poured into the tube; the disappearance of striations and the clearing of the field indicate when the previous solution has been completely displaced. The Pellet tube will accomplish a valuable saving of time in certain kinds of work, but it is usually advisable to limit its use to sugar solutions of approximately the same density; to displace a concentrated sugar solution with one that is exceedingly dilute, or vice versa, is attended with more or less risk of error.

Polarization Tube with Metal Jacket. For polarizing sugar solutions, where the temperature must be measured or controlled, a jacketed observation tube such as shown in Fig. 153 is recommended. This consists of an inner tube of glass or metal with a central opening, *c*, which can be used for filling and for inserting a thermometer; an outer mantle of brass or nickel surrounds the inner tube and is provided with nipples for inlet and exit of hot or cold water as may be desired.

An improved form of this tube is shown in Fig. 154. The funnel is closed with a ground-glass stopper, and the thermometer is ground into the stopper. A small capillary passes through the stopper to allow for expansion of the solution. The thermometer is bent outward at a right angle immediately above the stopper and then upward again, to

prevent obstruction in the beam of light which illuminates the scale. For the same reason the water inlet and outlet are mounted on the side, the outlet nipple at a level above the inlet nipple. A baffle plate is

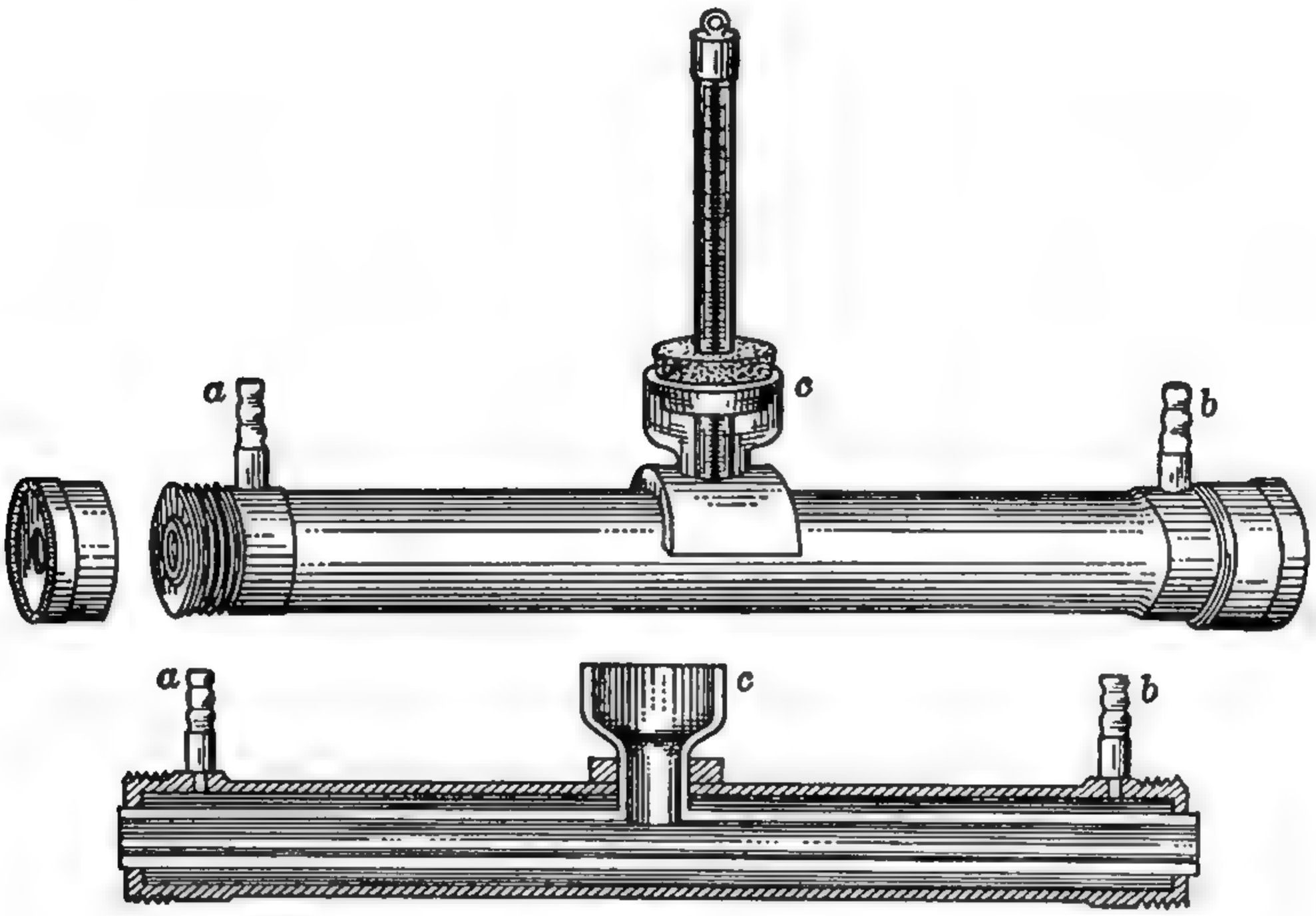
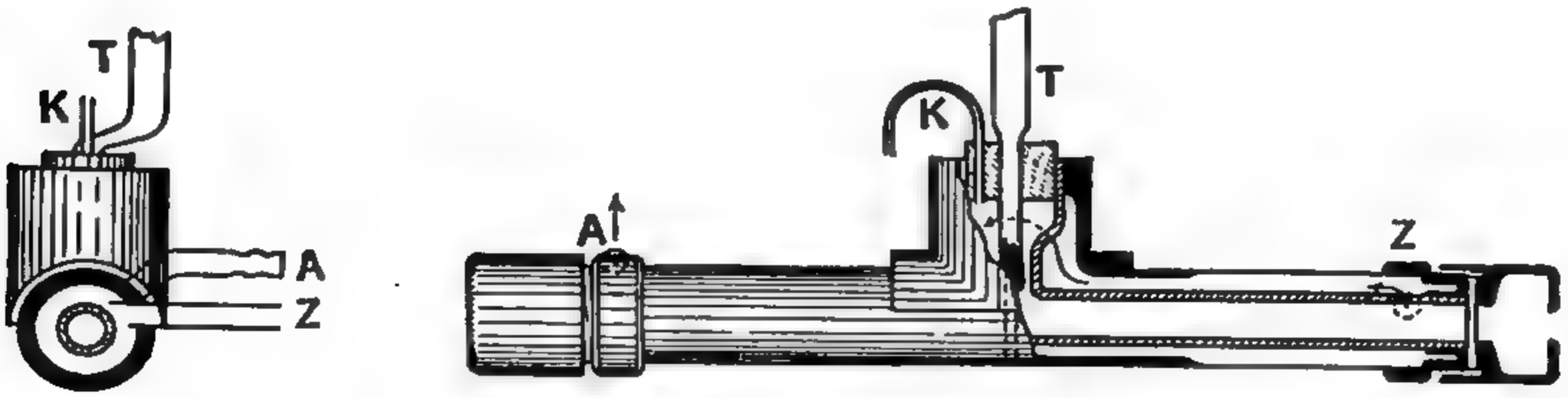


FIG. 153. Glass polariscope tube with metal jacket.

placed in the space between the tube and the jacket to assure better water circulation.

Tubes with a funnel opening in the center are also made without water jacket. They have the advantage for general work that the



(*Courtesy of Akatos, Inc.*)

FIG. 154. Improved form of jacketed polariscope tube.

cover glasses need to be removed only occasionally for cleaning, and that a thermometer may be inserted. But there is danger of evaporation if the tubes are allowed to stand for any length of time without

being stoppered, and without the water jacket there is no guarantee of uniform temperature throughout the tube.

For supplying water of constant temperature for observation tubes, the Zeiss apparatus described on p. 95 may be used. A form of water supply reservoir with stirrer, recommended by Landolt,[15] is shown in Fig. 155. The reservoir, which is insulated, is filled through the opening *A* with water to the desired level, indicated by the tube *D*. The water is heated by means of a burner to the desired temperature, shown by the thermometers at *C*, the heat being equalized by raising and lowering the stirrer *B*.

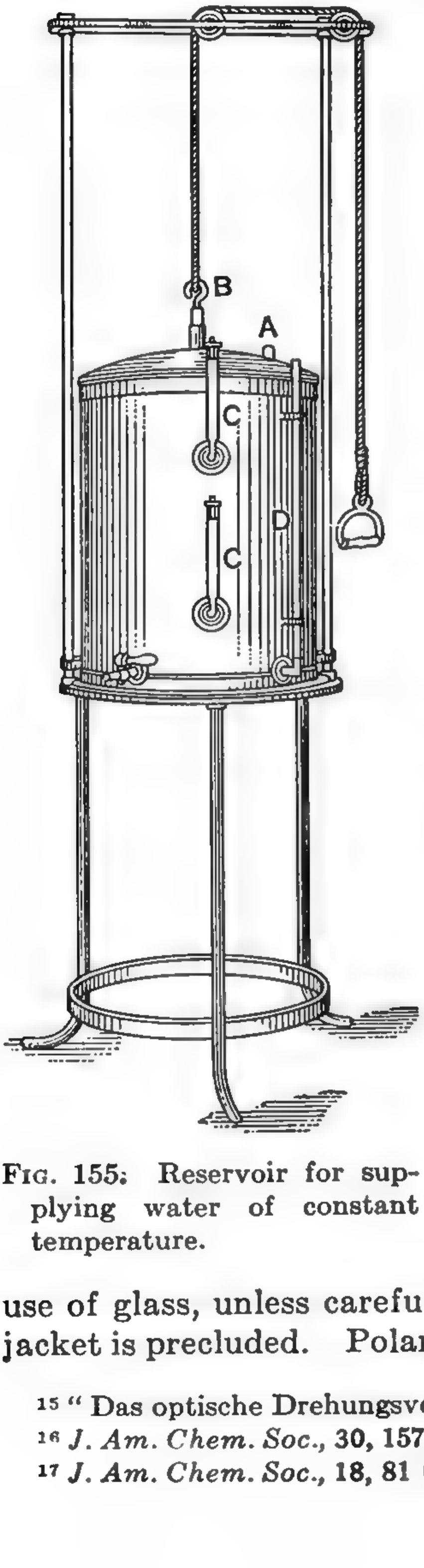


FIG. 155. Reservoir for supplying water of constant temperature.

A form of constant temperature bath designed by Hudson[16] is shown in Fig. 156. The mechanical stirrer not only secures an even temperature through the bath, but also acts as a rotary pump which creates a constant circulation of water as shown by the direction of the arrows.

Wiley's Desiccating Caps. When solutions are polarized at temperatures below the dew point of the atmosphere, the cover glasses of the observation tube must be protected against condensation of moisture by means of desiccating caps such as designed by Wiley[17] (Fig. 157). These are generally made of some non-conducting material such as hard rubber: they are closed at the end with a tightly fitted cover glass and contain a tube for holding calcium chloride or other desiccating substance.

Tubes for High-Temperature Polarization. When solutions are polarized at very high temperatures as at 87° C. (the point of inactivity for invert sugar) the use of glass, unless carefully annealed, for the inner tube of the water jacket is precluded. Polariscopic work at high temperature is generally

[15] " Das optische Drehungsvermögen," 2nd ed., p. 397, 1898.
[16] *J. Am. Chem. Soc.*, 30, 1572 (1908).
[17] *J. Am. Chem. Soc.*, 18, 81 (1896).

performed in jacketed tubes constructed entirely of brass or nickel, the inner surface of which has been gold plated. The length of a 200-mm. tube (20° C.) at 87° C. would be 200.107 mm. for glass and 200.255 mm. for brass, equivalent to a plus error of 0.054° V. and 0.128° V. respectively for solutions polarizing 100° V. in a 200-mm. tube.

A jacketed glass tube for high-temperature polarization has been designed by Bellingham and Stanley (Fig. 158).[18] In this tube the jacket is not made rigid, but in threaded parts separated by rubber rings which still make a tight joint after the tube expands. In case of breakage the jacket can be taken apart and a new glass tube inserted.

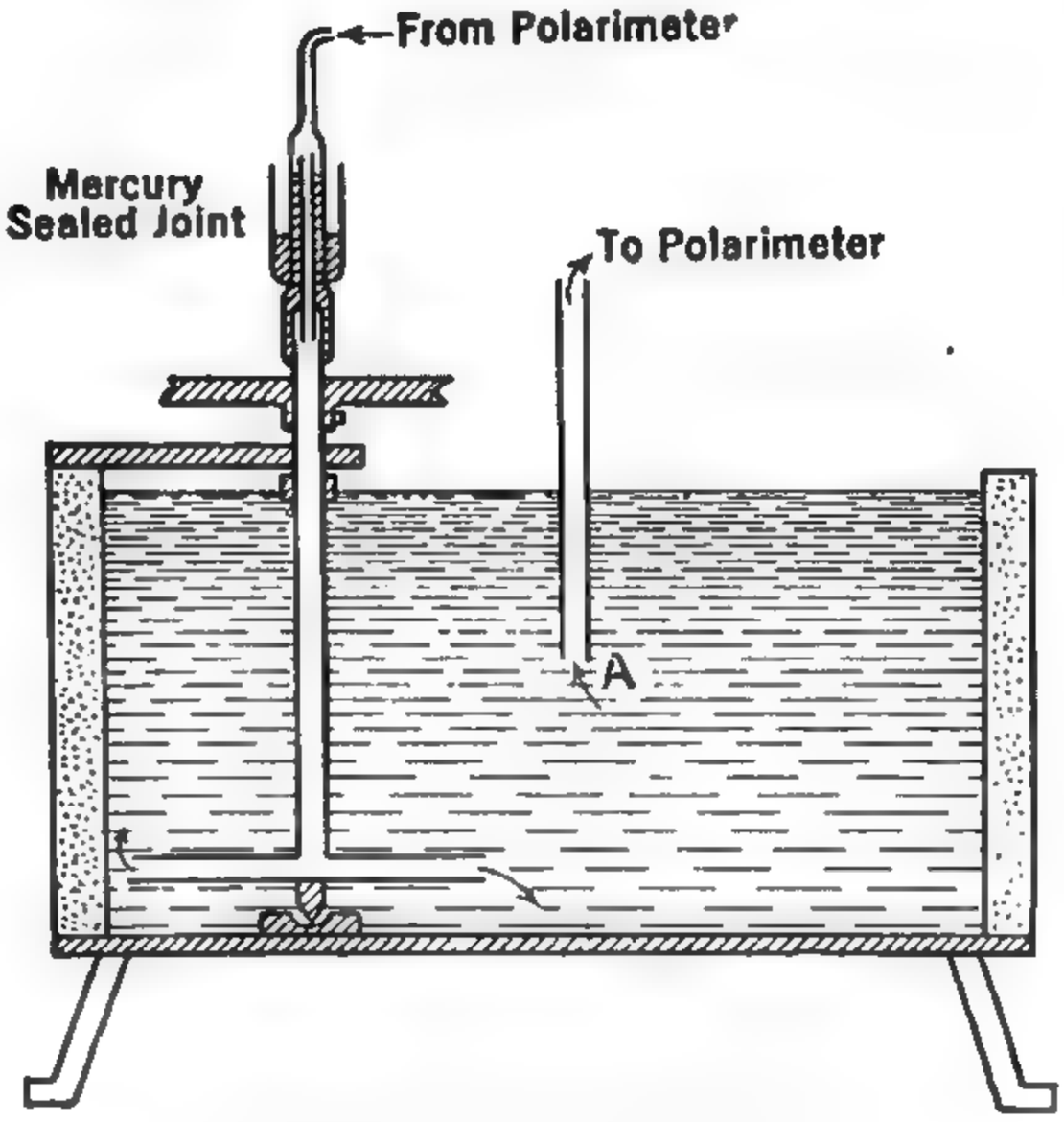
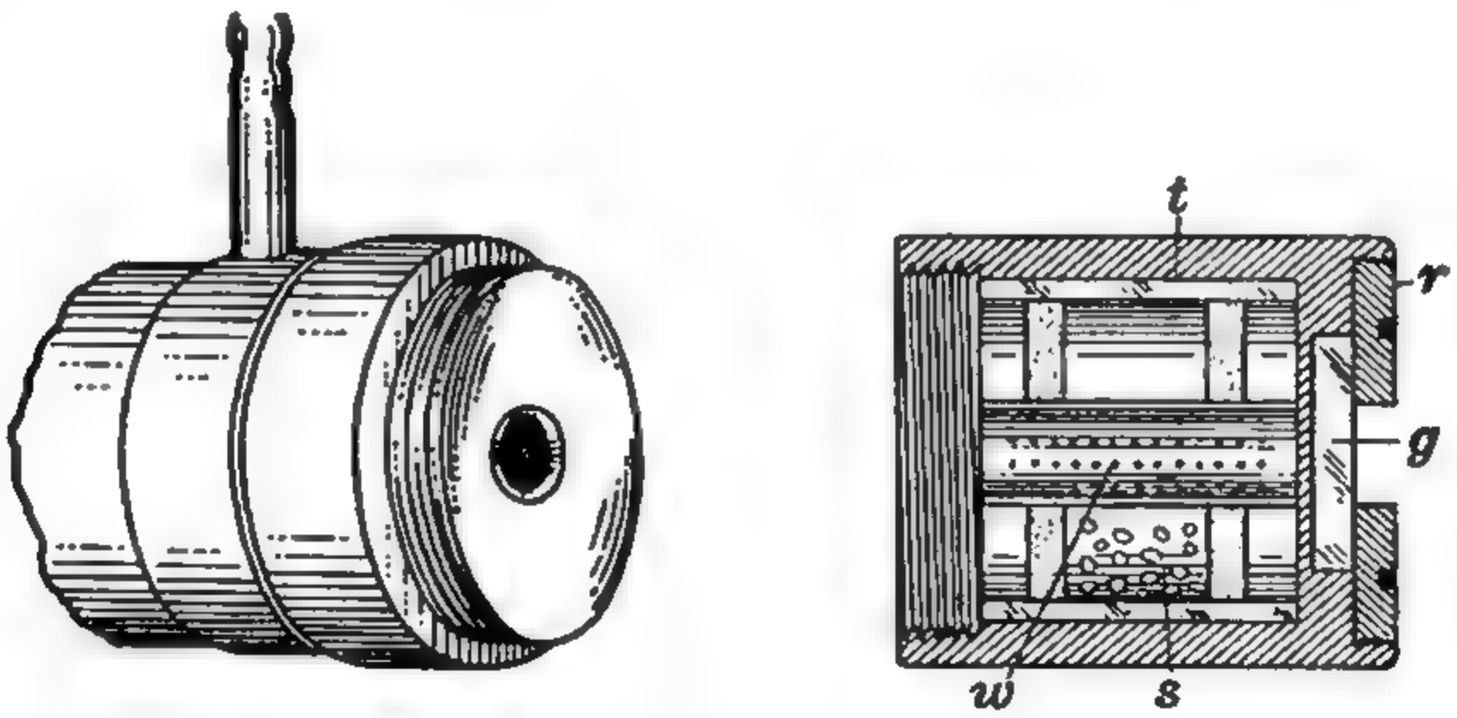


FIG. 156. Hudson's constant-temperature water bath.

The National Bureau of Standards has constructed a jacketed tube[19] (see Fig. 128, p. 220), made of "Invar" alloy, which has a negligible coefficient of expansion, so that no correction need be applied to the tube length in high-temperature



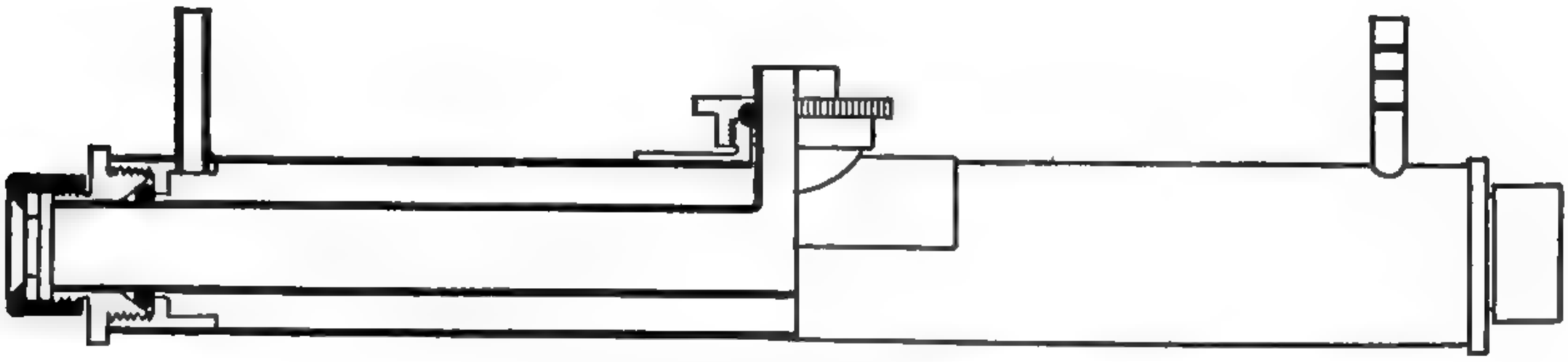
(I) (II)

FIG. 157. (I) Threaded cap of polariscope tube. (II) Desiccating cap which screws on over threads of (I); *t*, removable glass tube containing desiccating substance *s*; *w*, inner perforated metal tube; *g*, cover glass held in position by threaded disk *r*; the disk is unscrewed by inserting a spanner in the two holes marked in black.

[18] Pellet, *Intern. Sugar J.*, 19, 76 (1917).

[19] Sold by Akatos, Inc., 55 Van Dam Street, New York, N. Y.

polarizations. The inside of the tube itself is plated with gold or chromium, and the outside of the jacket is also chromium plated. Perforated baffles are placed in the jacket to insure uniform circulation. The nipples for the water inlet and outlet are placed at an angle so that they do not interfere with the beam of light used for illuminating the scale.



(Courtesy of Bellingham and Stanley.)

FIG. 158. Jacketed glass tube for high temperature polarization.

Yoder's Volumetric Polariscope Tube. A volumetric polariscope tube is convenient for certain kinds of saccharimetric work. A tube of this description, designed by Yoder, is shown in Fig. 159.

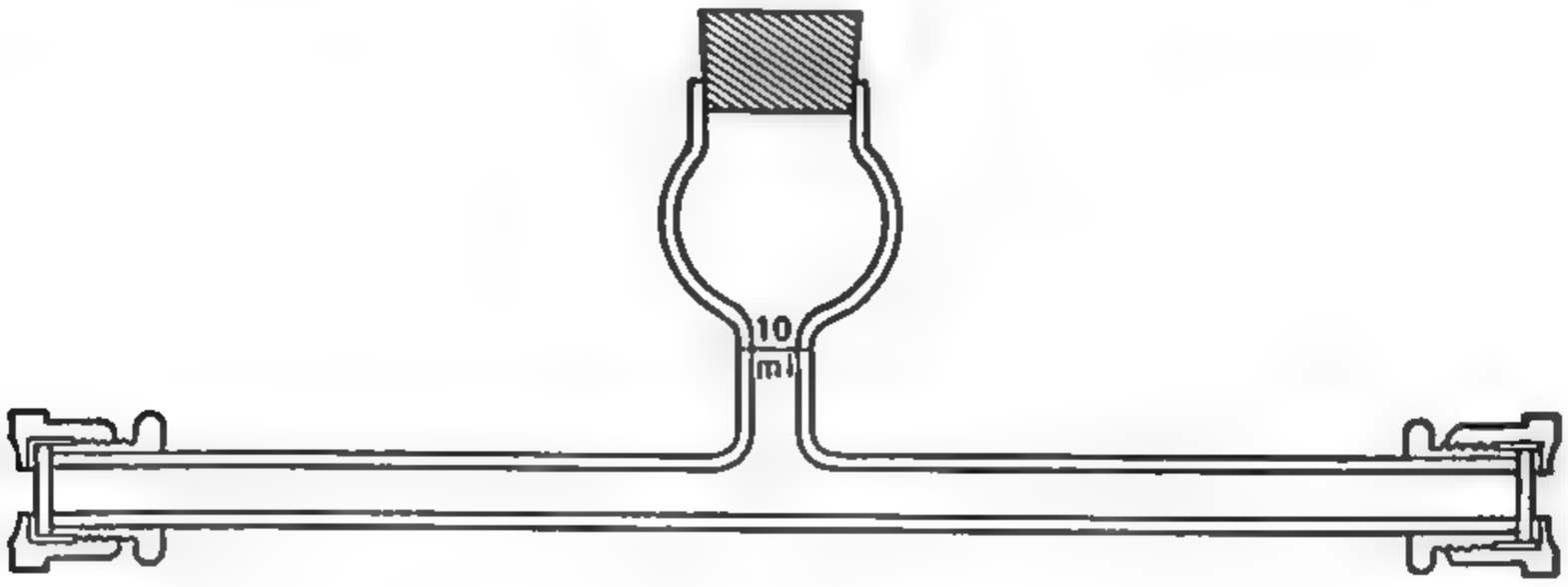


FIG. 159. Yoder's volumetric polariscope tube.

The capacity of the tube to the graduation mark upon the neck is 10 ml. By varying the length and diameter the tubes can be adjusted to any convenient volume.

When only small quantities of material are available, as in certain biochemical investigations, special tubes with small bore, or even with capillary bore, holding as little as 0.1 ml. of liquid, are employed. To avoid errors caused by reflection from the walls of these tubes, Naumann[20] recommends that they be made of black glass and etched inside with hydrofluoric acid.

[20] *Biochem. Z.*, **211**, 239 (1929).

BALANCES FOR POLARISCOPIC WORK

For the operations of weighing in saccharimetric work three types of balances are required, an analytical balance, a so-called sugar balance, and a balance for coarse weighing.

The analytical balance should have a capacity of 200 g. and with this load be sensitive to 0.1 mg. Such a balance is required for all analytical processes, for determination of specific rotations, for calibration of flasks, weighing of pycnometers, and all other operations where accuracy is essential. A balance of the type shown in Fig. 28 will answer for this purpose. With this balance a set of accurate analytical weights (including one 100-g. weight) will be needed.

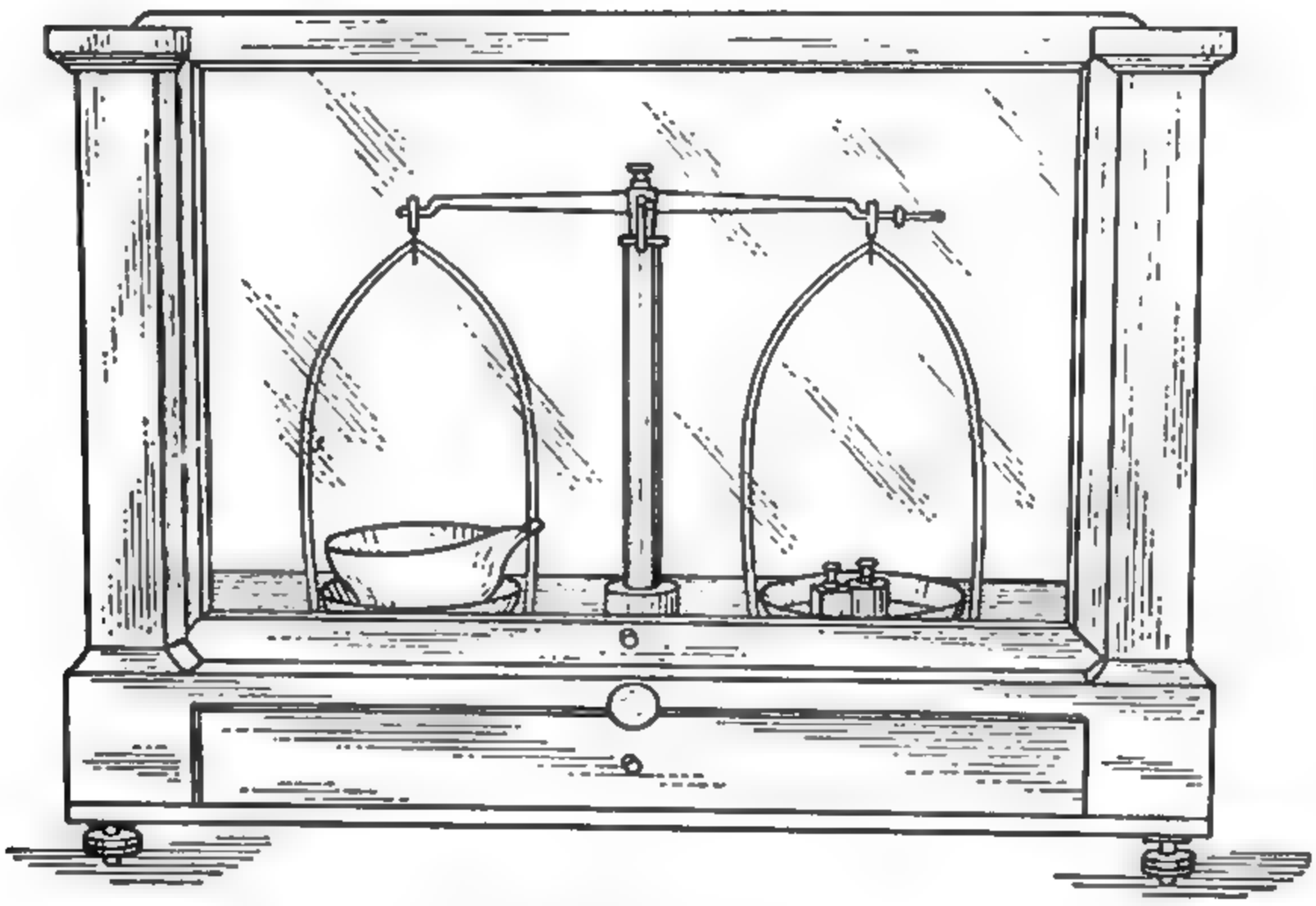
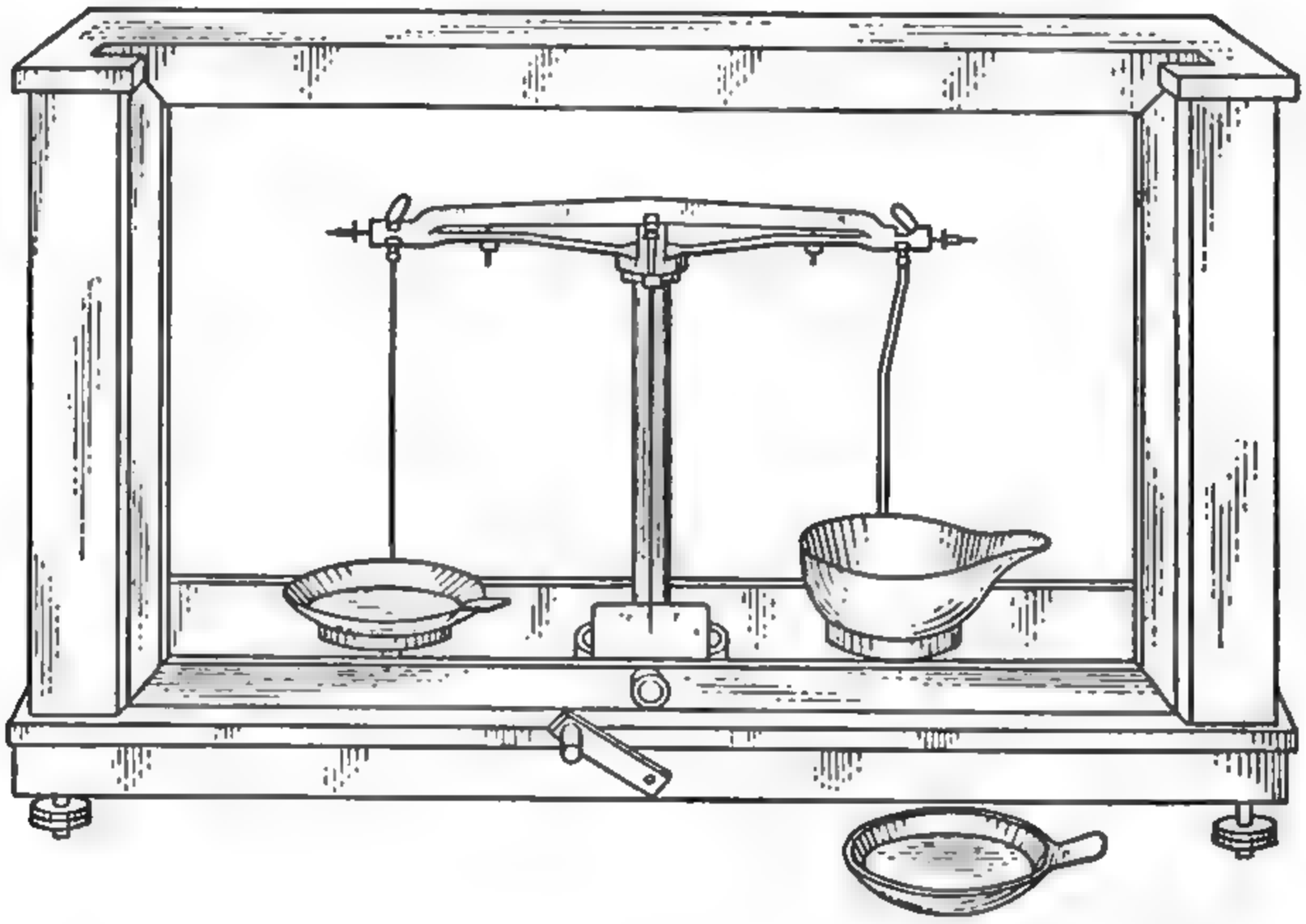


FIG. 160. Sugar balance.

In addition to the above a less delicate balance, sensitive to 2.5 mg. with a load of 250 g., will be required for the rapid weighing of definite amounts of sugar, molasses, and other products for ordinary saccharimetric work. For saccharimeters employing a normal weight of 26 g., 0.01° sugar scale corresponds to 0.0026 g. sucrose in 100 ml. Since the majority of saccharimeters can be read only to 0.05° it is evident that weighing within 5 mg. is sufficiently accurate for ordinary purposes of saccharimetry. The weighing out of normal weights of sugars, etc., for saccharimeters should not be done upon an analytical balance; the errors due to evaporation from moist substances during the slower adjustment of the analytical balance will usually exceed any advantage in greater accuracy of weight. A so-called " sugar balance " of the type shown in Fig. 160 answers very well for this kind of work. This balance may also be used for the weighing out of chemicals

for making up solutions of reagents. A set of weights should be provided for approximate weighing, and also the normal weights belonging to the saccharimeter.

A special sugar balance, shown in Fig. 161, has been designed by Bates. The pan on which the sugar dish is placed is made small enough so that particles of sugar will not fall on it during the weighing operation. The bow consists of a single arm only, placed in the rear. This facilitates the placing and removal of the sugar dish without hitting against the bow. The knife edges and planes are made of agate instead of steel, which is liable to rust. The release



(Courtesy of Eimer and Amend.)

FIG. 161. Bates's sugar balance.

and arrest of the beam are so constructed that all edges are free from the bearings when the balance is at rest; this protects the balance from injury during weighing operations. The capacity of the balance is 200 g., with a sensitivity of 1 mg. Owing to this high sensitivity the balance is rather slow, and this is inconvenient in a busy routine laboratory when large numbers of samples must be handled in a minimum of time, aside from the fact that the sample may lose or gain weight while it is being weighed.

The Mohr cubic centimeter normal and half-normal weights (26.048 g. and 13.024 g.) are usually furnished in a cylindrical form, the true cubic centimeter weights (26.000 g. and 13.000 g.) in a cubical form (Fig. 169), and the new weights adopted at the Eighth Session of the International Commission for Uniform Methods of Sugar Analysis, 1932 (26.026 g. and 13.013 g.), in hexagonal form, as suggested by

Browne and Balch[21] (Fig. 162), the shape of the weight thus guarding against confusion. Normal weights, which are in constant use, should

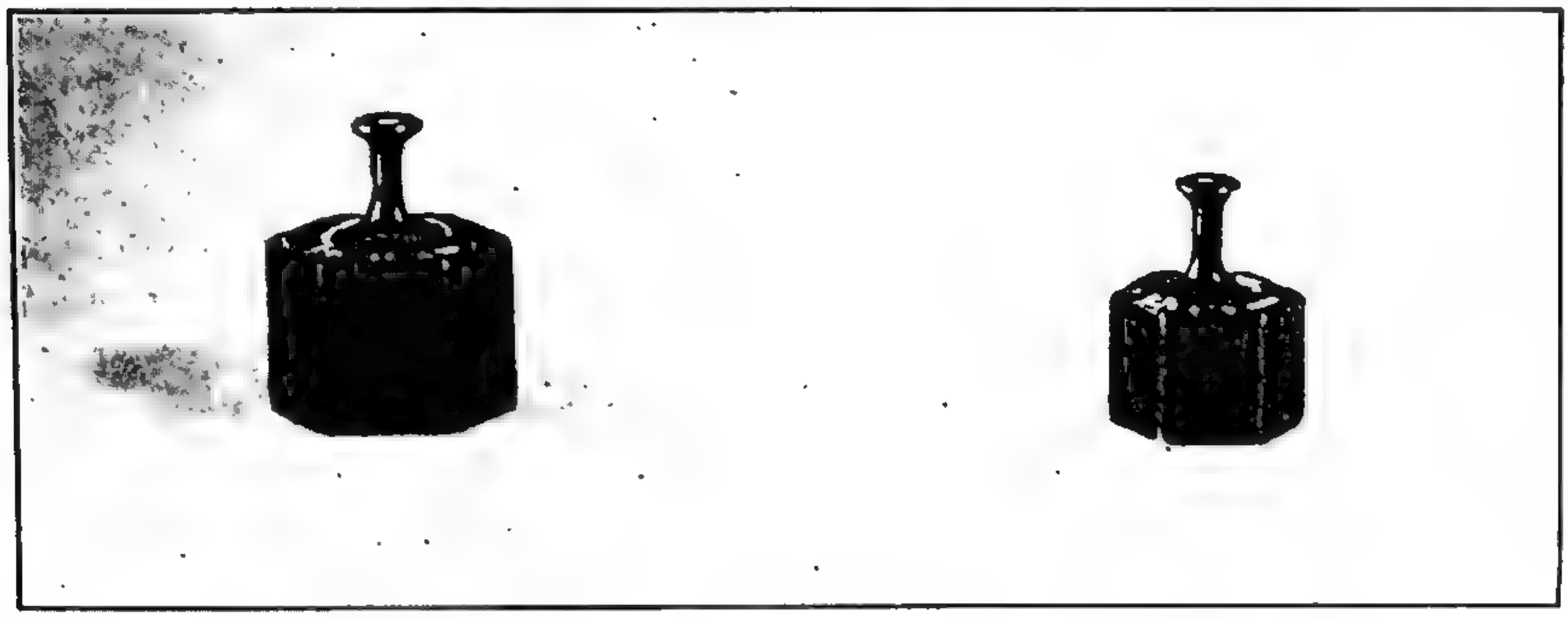


FIG. 162. Hexagonal normal sugar weights of 26.026 g. (left) and 13.013 g. (right).

be tested frequently upon the analytical balance against losses in weight through wear. If a deficiency exceeding 1 mg. is noted, the stem of the weight should be unscrewed and a small piece of tin or

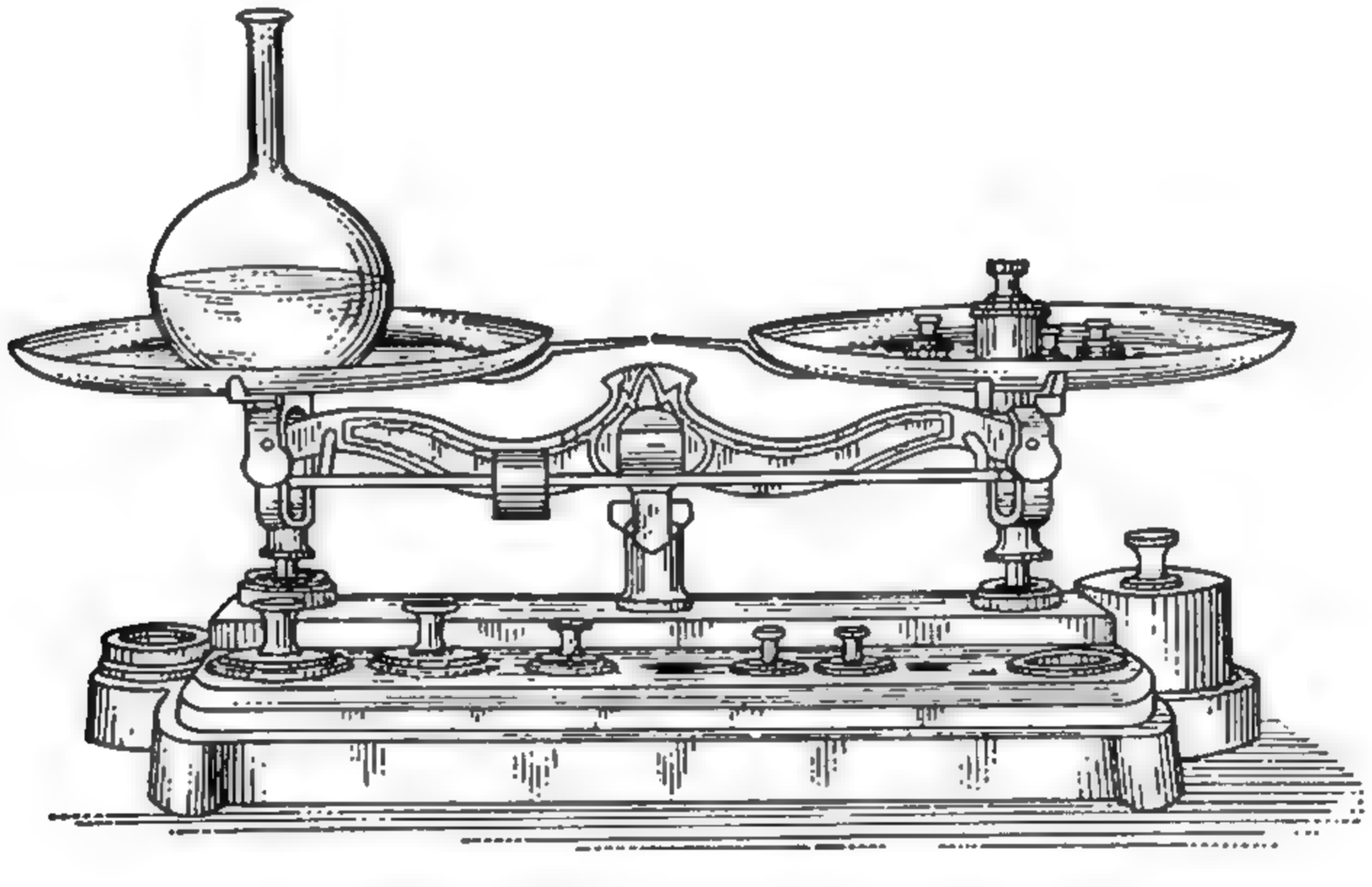


FIG. 163. Metric solution balance.

aluminum foil be placed in the cavity sufficient to bring the weight up to its proper value.

In addition to the two kinds of balances just described a heavy balance or scale for weighing out material in bulk, preparing large quanti-

[21] *Ind. Eng. Chem., Anal. Ed.*, 5, 283 (1933).

ties of reagents, etc., will be required. A metric solution scale with sliding counterpoise such as is shown in Fig. 163 is very good for this purpose. A set of weights up to 5 kg. should also be provided for coarse weighings.

FLASKS FOR POLARISCOPIC WORK

For the preparation of sugar solutions in polarimetric and saccharimetric work various flasks have been devised of different shape and construction.

Flasks for Solution by Weight. When sugar solutions are made up according to percentage a glass-stoppered flask of the form shown as No. VI in Fig. 164 is recommended. The flask, which is supplied in many sizes, need not be graduated. Before using, it is thoroughly cleansed and dried, and then weighed. The approximate quantity of substance to be examined is then transferred to the flask, and the flask, after being stoppered, is reweighed. The approximate amount of distilled water or other solvent is then added and the flask and contents reweighed as before. The percentage of substance in solution is then readily calculated from the weight of substance taken and the combined weights of substance and solvent. The flask should not be filled too full; sufficient space should be left for gentle rotation of the liquid while effecting solution. The flask should always be kept stoppered to prevent evaporation.

Reduction of Solution Weights to Vacuò. For very accurate physical measurements the weights taken in air must be reduced to vacuo, since a substance weighed in any medium loses in weight an amount equal to that of the medium displaced. If W is the true weight of a substance of density D, in vacuo, then the volume of substance is W/D, and if s is the density of the air at the time of weighing, the loss in weight of the substance in air will be sW/D. Similarly if P is the value of the weights in vacuo and d is the density of their material then the loss of the weights in air will be sP/d. The equilibrium upon the pans of the balance between substance and weights in air will then be represented by the equation

$$W - \frac{sW}{D} = P - \frac{sP}{d}$$

whence

$$W = P\,\frac{1 - \frac{s}{d}}{1 - \frac{s}{D}}\,.$$

The mean value 0.0012 g. may be taken as the weight of 1 ml. of air without sensible error. When brass weights are used ($d = 8.4$), the weights in vacuo of glass, water, and sugar are found as follows: for glass ($D = 2.5$) the weight in vacuo equals 1.000337 times the weight in air; for water at 20° C. ($D = 0.998234$) it equals 1.001061 times the weight in air; and for cane sugar ($D = 1.59$) it equals 1.000612 times the weight in air. The following example will illustrate the method of application.

Weight of flask + sugar in air	35.2326 g.
Weight of flask alone in air	25.1240 g.
Weight of sugar in air	10.1086 g.
Weight of sugar in vacuo = 10.1086 × 1.000612 =	10.1148 g.
Weight of flask + sugar + water in air	95.3055 g.
Weight of flask + sugar in air	35.2326 g.
Weight of water in air 20° C.	60.0729 g.
Weight of water in vacuo = 60.0729 × 1.001061 =	60.1366 g.
Weight of sugar + water in vacuo =	70.2514 g.

Per cent sugar in solution from weights in air = 14.403 per cent.
Per cent sugar in solution from weights in vacuo = 14.398 per cent.

It will be noted that the difference is exceedingly slight, so that weighing in air is sufficiently exact for all operations except those demanding extreme accuracy.

Volumetric Sugar Flasks. When solutions of dissolved sugars are made up to a definite volume before polarization, a carefully calibrated volumetric flask must be used; such flasks are supplied in a variety of forms and sizes. If solutions are polarized immediately after making up to volume, as is usual, it is not essential that the flask be fitted with a glass stopper.

Volumetric flasks for sugar work are made in 10-, 20-, 25-, 50-, 100-, 200-, and 250-cc. (ml.) sizes; 300-, 500-, and 1000-cc. (ml.) flasks are also occasionally used. For certain kinds of work, where volume of insoluble matter is allowed for, flasks of irregular capacity are used, as 100.5-ml., 201.0-ml., etc., for polarization of sugar-beet pulp.

A few of the more ordinary forms of sugar flask are shown in Fig. 164. These may be obtained of any desired capacity. Small stoppered flasks similar to No. I are convenient for preparing solutions when only small amounts of substance are available. Kohlrausch's sugar flask (No. IV) with enlarged top is convenient for transferring substances like filter press cake and is also desirable for materials which produce much foam, like beet molasses. It can be obtained in various sizes

and, if desired, with ground-glass stopper. The Stift flask is similar to the Kohlrausch flask, but the neck of the flask above the mark widens gradually toward the top, instead of being cylindrical. A further improvement for certain purposes is the Mann flask,[22] which is flared out above the mark as in the Kohlrausch flask, but then narrows again so that the mouth can be readily closed with the finger and the flask thoroughly shaken without danger of spilling. Sugar flasks with double graduation (No. III) for one-tenth dilution are useful for the methods of inversion; they are supplied in 25–27.5-, 50–55-,

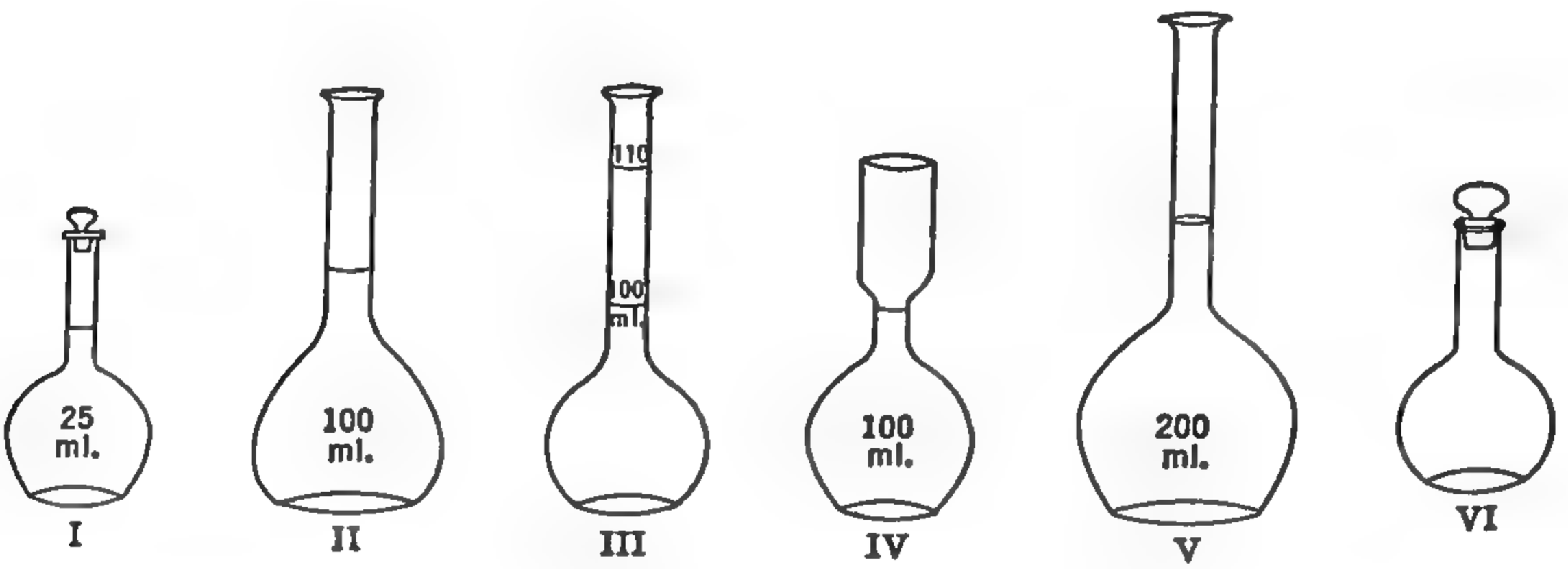


FIG. 164. Types of flasks for polariscopic analysis.

100–110-, and 200–220-ml. sizes. If these flasks are made with a wide neck, the accuracy of completing the volume to each mark suffers. If they have a narrow neck, the latter disadvantage disappears, but the neck must be made unduly long. The Stephan flask[23] overcomes both difficulties by the use of a narrow neck, with a bulb between the two graduations.

Specifications for Sugar Flasks. In the selection of sugar flasks the following requirements of the United States Bureau of Standards for volumetric flasks will be found useful.

The material should be the best quality of glass, transparent, and free from bubbles and striae. It should have small thermal hysteresis and should adequately resist chemical action. All flasks should be thoroughly annealed before being graduated.

The cross section of the neck must be circular, and the shape of the flask must be such as to admit of complete emptying and drainage from the whole interior surface at the same time. The bottom of the flask should be slightly concave upward, and should be of sufficient size to enable the flask to stand

[22] *Intern. Sugar J.*, **37**, 229 (1935).
[23] *Arch Suikerind.*, **34**, 1149 (1926).

on a surface inclined at an angle of 15 degrees to the horizontal. The neck must be cylindrical for at least 1 cm. on each side of every graduation mark, but may be enlarged in the form of a bulb between graduation marks (for example, Giles flasks). At the graduation mark the inside diameters of the neck of the flask must be within the following limits:

Capacity of flask (in ml.) up to and including.....	25	50	100	200	250	500	1000	2000	3000	4000	5000	6000
Maximum diameter (in mm.)	8	10	12	13	15	18	20	25	30	35	40	45
Minimum diameter (in mm.)	6	6	8	9	10	12	14	18	20	22	25	30

The graduation marks must be of uniform width, finely but distinctly etched, must be perpendicular to the axis of the flask, and must extend completely around the neck.

On flasks having a capacity of 100 ml. or less the graduation mark shall be not less than 3 cm. from the upper end nor less than 1 cm. from the lower end of the neck; and on flasks having a capacity of more than 100 ml. the graduation mark shall be not less than 6 cm. from the upper end nor less than 2 cm. from the lower end of the neck.

A very desirable 100-ml. flask for saccharimetric work is that shown in No. II, Fig. 164, and in Fig. 169, designed for use in the Custom House Laboratories of the United States Treasury Department. The pear-shaped body with its low center of gravity gives the flask greater stability than a spherical form. According to the regulations of the Treasury Department " the flasks shall have a height of 130 mm.; the neck shall be 70 mm. in length and have an internal diameter of not less than 11.5 mm. and not more than 12.5 mm. The upper end of the neck shall be flared, and the graduation marks shall be not less than 30 mm. from the upper end and 15 mm. from the lower end of the neck." With this size of flask the base of the thumb can cover the mouth and the fingers of the same hand easily enclose the bottom — a feature of great convenience when mixing the contents after making up to volume.

Calibration of Sugar Flasks. Sugar flasks are graduated to contain 100 ml. at 20° C. or 100 Mohr cc. at 17.5° C. and should be calibrated before using in the following manner. The flask to be tested is first thoroughly cleaned and dried, then weighed empty at the temperature of standardization, and then again when filled to the mark with distilled water at the standard temperature. The distilled water should be boiled just before using, in order to expel dissolved air, and then cooled. Special care is necessary in adjusting the meniscus to the graduation mark; the lowest point of the curve when viewed against a white surface should just touch the level of the

graduation mark, the latter appearing to the eye in proper position as a straight line and not as an ellipse. Figure 165 indicates the proper method of adjustment. The inside of the neck above the meniscus should be wiped perfectly dry with filter paper before reweighing; air bubbles should not be allowed to adhere to the walls of the flask during calibration.

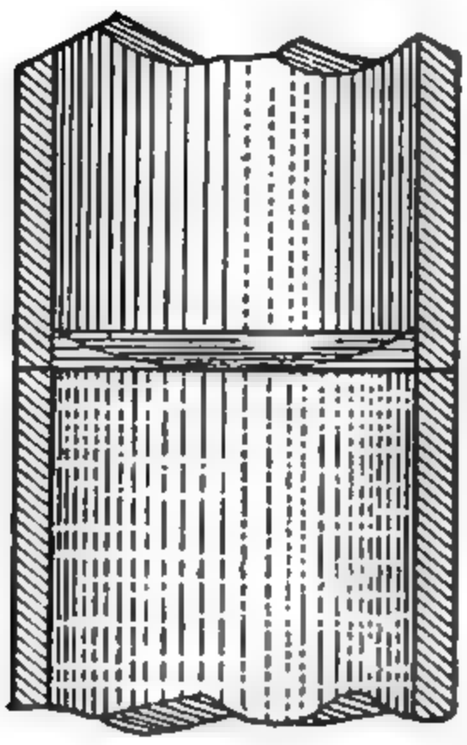


FIG. 165. Showing proper adjustment of meniscus.

Volumetric 100-cc. sugar flasks graduated according to the Mohr system should contain 100 g. of distilled water at 17.5° C., when weighed in air against brass weights; 100-ml. flasks, graduated according to true cubic centimeters, should contain 100 g. of distilled water at 4° C. when weighed in vacuo or 99.7176 g. at 20° C. when weighed in air with brass weights. (Weight in vacuo of 100 ml. water at 20° C. is 99.8234 g. and weight in air (p. 255) is 99.8234 ÷ 1.001061 = 99.7176 g.) The grams of water contained by the flask at 20° C. plus the correction 0.282 will give the volume in true cubic centimeters.

The limits of error allowed by the National Bureau of Standards for volumetric flasks are the following:

Capacity	Limit of Error
ml.	ml.
2000	0.5
1000	.3
500	.15
300	.12
200	.1
100	.08
50	.05
25	.03
10	.01

The limit of error allowed above for 100-ml. sugar flasks is, however, too high; the error of graduation should not exceed 0.05 ml., and careful manufacturers can conform to this requirement without trouble. A lot of 200 sugar flasks purchased by the New York Sugar Trade Laboratory showed upon calibration the errors given in the table on p. 259.

It is seen that 99 per cent of the flasks were correct within 0.05 ml. and that over 95 per cent were correct within 0.04 ml.

Error in Volume	Number of Flasks	Percentage
Between 0.00 ml. and 0.01 ml.	65	32.50
Between 0.01 ml. and 0.02 ml.	56	28.00
Between 0.02 ml. and 0.03 ml.	43	21.50
Between 0.03 ml. and 0.04 ml.	27	13.50
Between 0.04 ml. and 0.05 ml.	7	3.50
Between 0.05 ml. and 0.06 ml.	2	1.00
	200	

FUNNELS AND CYLINDERS

In filtering sugar solutions for polarization short-stemmed funnels and cylinders of any of the forms shown in Fig. 166 will be found convenient. The funnels and filters should be of sufficient size to retain 100 ml. of solution. In the routine testing laboratory the breakage of glass funnels is quite high, and for this reason the New York Sugar

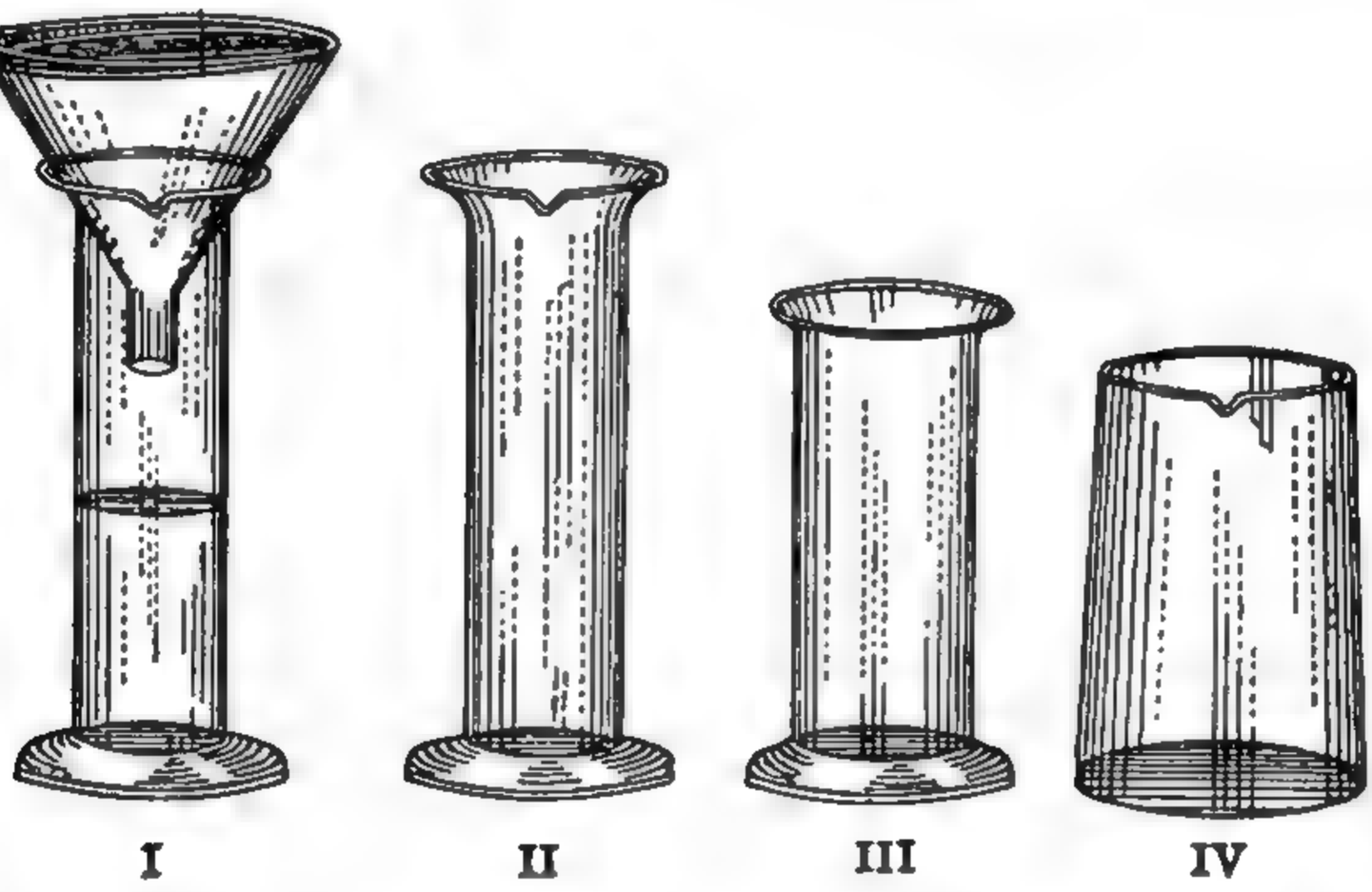


FIG. 166. Types of filtering cylinders for polariscopic analysis.

Trade Laboratory uses hard-rubber funnels of ½-pint capacity. They have been found very satisfactory. The funnels should be covered with large watch glasses during filtration to prevent evaporation. Tall narrow filtering cylinders (Nos. I and II, Fig. 166) are preferred by some chemists for the reason that the least surface of filtered liquid is exposed to evaporation. The small-lipped filtering jars (No. IV, Fig. 166) are more convenient, however, for filling tubes, and if covered by

funnels and watch glasses will not allow sufficient evaporation, during the necessary time of filtration, to cause any appreciable error in the polariscope reading. The New York Sugar Trade Laboratory uses ordinary screw-top preserve jars, of 1-pint capacity, discarding the screw tops. These are very economical and serve the purpose well.

MOUNTING OF POLARISCOPES AND CARE OF APPARATUS

If the circumstances permit, polariscopes should always be mounted in a separate room or compartment, where there is no danger of corrosion from the action of fumes or vapors. The polarizing compartment should be well ventilated and easily darkened; lamps and burners for illumination should be placed upon the opposite side of a wall or partition.

In the New York Sugar Trade Laboratory the polariscope cabinet (Fig. 167) constitutes a section of the constant-temperature room. The sides of the cabinet are enclosed by dark curtains, which, when drawn, leave a space of 2 to 3 feet at the bottom. This arrangement allows free circulation of air, and the presence of several observers in the cabinet does not affect the temperature.

Where room is not available for a separate compartment, the polariscopes may be mounted in a large box in a dark corner of the laboratory as shown in Fig 168.

The table supporting polariscopes should be of solid construction. By placing the table upon rubber cushions and setting the polariscopes upon rubber mats, vibration of the instruments and consequent disturbance of the zero point will be largely obviated.

It is essential in saccharimetric work that all apparatus be kept scrupulously clean. The more delicate optical parts of polariscopes, such as polarizer, analyzer, and quartz compensation, are enclosed, in the most modern apparatus, in dust-proof housings, and very rarely require to be disturbed. The diaphragm glasses (*A* and *P*, Fig. 133) at each end of the polariscope trough are the parts which require most attention. Drops of solution, accidentally adhering to the polariscope tubes, are occasionally splashed against the diaphragm glasses. The diaphragms, which either screw or slide into position, should be examined frequently and the glasses wiped free of dirt and dust particles. A paper napkin will be found very suitable for cleaning diaphragm glasses, eyepieces, and other exposed optical parts.

The troughs of polariscopes in the hasty round of routine frequently become soiled from contact with wet tubes or spilled liquid. They should be wiped frequently with a damp cloth, and the metal surface should be kept smooth and clean.

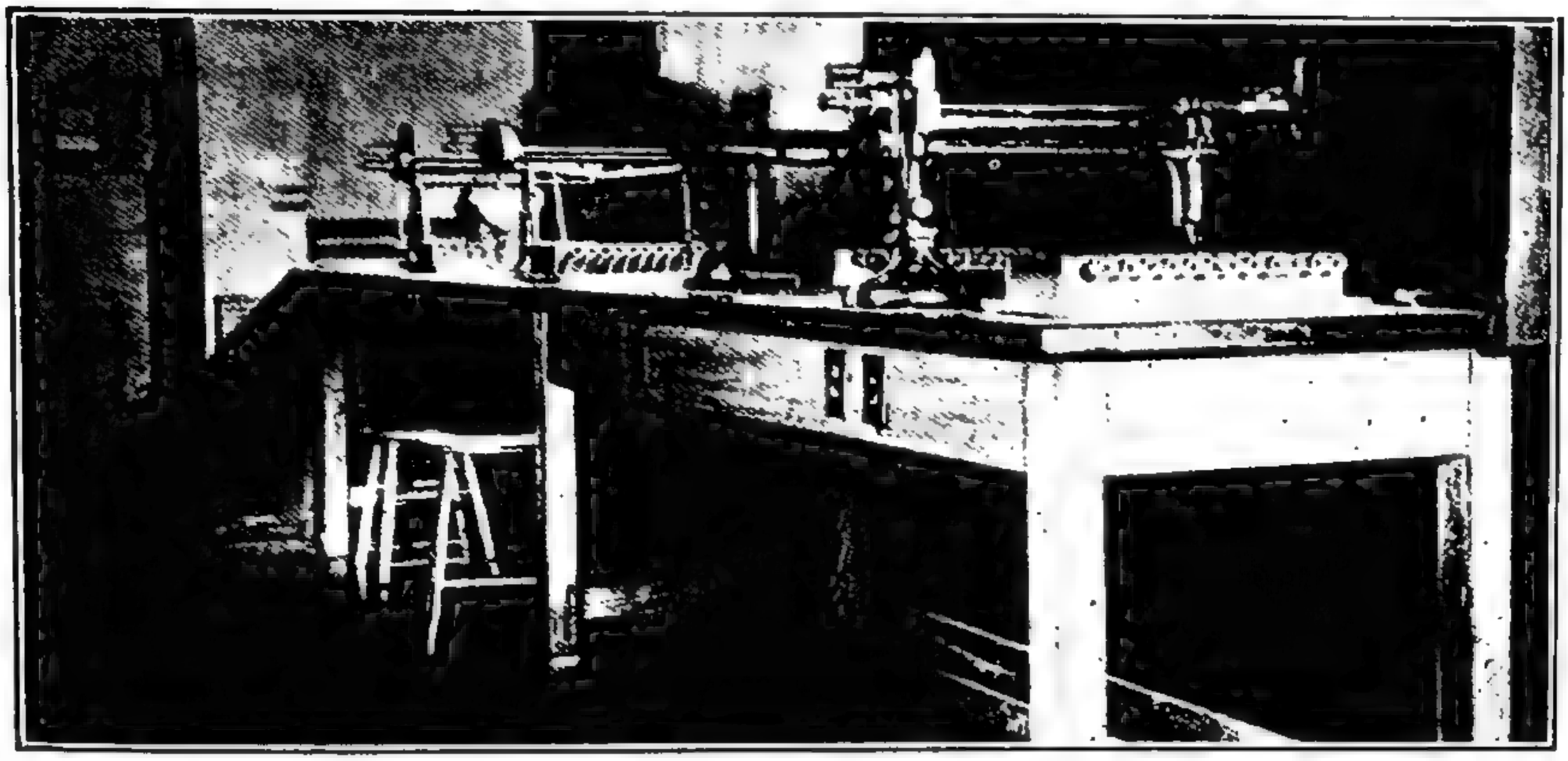


FIG. 167. Cabinet for constant temperature polarization (New York Sugar Trade Laboratory).

FIG. 168. Portable polariscope cabinet with section of side removed

The bichromate cell should be examined frequently, and the solution replenished as soon as bubbles begin to form; otherwise their appearance may obscure the field. If a light filter of glass is used, instead of the bichromate cell, this also should be kept clean.

When the polariscope is not in use, the trough should be closed and the instrument kept covered.

Strict cleanliness must also be observed in the use of polariscope tubes, flasks, and other accessories. In handling and carrying observation tubes a portable rack of the form shown in Fig. 168 will be found convenient.

Where sugar solutions are clarified with lead subacetate, the walls of flasks, cylinders, funnels, and tubes become coated in time with a thin white film of lead carbonate. A good solvent for this coating is a warm solution of sodium hydroxide and Rochelle salts, such as is used in preparing Fehling's solution. Hydrochloric or nitric acid may also be used for removing the deposit. After thorough rinsing in clean water, tubes, flasks, funnels, and cylinders should be allowed to drain and dry upon racks.

CHAPTER VIII

SPECIFIC ROTATION OF SUGARS

In the previous chapters the principles which underlie the construction and operation of polariscopes were described; it is now desired to study the application of these principles to some of the problems of sugar analysis.

The polarizing power of a sugar is expressed as specific rotation, or specific rotatory power, by which is meant the calculated angular rotation which a solution, containing the mass of 1 g. active substance in a volume of 1 ml., and 1-dm. long, gives to the plane of polarized light. The specific rotation, indicated by the expression $[\alpha]$, can easily be calculated from the angular rotation a of the solution of substance by means of the equation $[\alpha] = \frac{100\,a}{c \times l}$, in which c is the concentration of substance (grams mass per 100 ml. solution) and l the length of the observation tube in decimeters. Instead of the foregoing we may use the equation $[\alpha] = \frac{100\,a}{p \times d \times l}$, in which p is the percentage of substance in solution (parts by weight in 100 parts by weight of solution) and d is the true density of the solution. ($p \times d = c$ in previous equation.)

The angular rotation, as shown below, depends upon the wavelength of the light employed. Sodium light is the illumination most used for polariscopic measurements, and, as the bright yellow line of sodium is designated the D line of the solar spectrum, the expression $[\alpha]$ for sodium light is written $[\alpha]_D$. Specific rotation for the mean yellow ray j (now no longer used) is written $[\alpha]_j$. The temperature at which the specific rotation is taken is also usually affixed. Thus: the symbol for specific rotation using sodium light at 20° C. is written $[\alpha]_D^{20}$.

The method of calculating specific rotation may best be understood by an example; 20 g. of cane sugar weighed in vacuo and dissolved to 100 ml. gives an angular rotation for sodium light of +53.2° in a 400-mm. tube at 20° C. Substituting these values in the equation $[\alpha] = \frac{100\,a}{c \times l}$, we obtain $[\alpha]_D^{20} = \frac{100 \times 53.2}{20 \times 4} = +66.5$, the specific rotation of sucrose for the given concentration.

To calculate specific rotation from the reading of a saccharimeter, the scale divisions of the latter must first be converted to angular degrees by means of the appropriate factor. Thus: 15 g. of sucrose dissolved to 100 ml. gave a reading of +57.7 in a 200-mm. tube using a Ventzke scale quartz-wedge saccharimeter. Since 1° V. = 0.34657 angular degrees (p. 228) then

$$[\alpha]_D \text{ sucrose} = \frac{100\ (0.34657 \times 57.7)}{15 \times 2} = +66.6$$

Effect of Kind of Light upon Specific Rotation of Sugars

Mention has been made of the influence of wavelength of light upon specific rotation. In Table XXXI a comparison was given of the rotations of quartz and sucrose for light of different wavelengths, and it was shown that as the wavelength decreases the polarizing power of sucrose increases. In the following table the specific rotations of nine different sugars are given for light of different wavelengths in the red, yellow, green, blue, indigo, and violet parts of the spectrum, according to measurements by Grossmann and Bloch.[1] The specific rotations for yellow sodium light, $[\alpha]_D$, the standard values of comparison, are printed in heavier type.

TABLE XXXVIII

Specific Rotations of Nine Sugars for Different Wavelengths of Light

Sugar	Concentration, g. 100 ml.	Red (*r*) 656 mμ	Yellow (*y*) 589 mμ	Green (*g*) 535 mμ	Blue (*b*) 508 mμ	Indigo (*i*) 479 mμ	Violet (*v*) 447 mμ	Dispersion Coefficient *v/r*
Xylose....	0.866	+ 13.28	+ 18.19	+ 21.08	+ 24.50	+ 27.70	+ 31.94	2.41
Rhamnose.	6.948	+ 7.08	+ 8.37	+ 10.27	+ 11.11	+ 12.84	+ 14.38	2.03
Galactose.	5.603	+ 60.80	+ 80.72	+ 99.63	+116.76	+131.84	+152.90	2.51
Glucose...	4.500	+ 41.89	+ 52.76	+ 65.35	+ 73.61	+ 83.88	+ 96.62	2.30
Fructose..	4.500	− 76.39	− 90.46	−107.21	−136.85	−151.11	−166.55	2.18
Sucrose...	4.275	+ 53.18	+ 66.50	+ 82.25	+ 91.53	+104.24	+121.63	2.29
Lactose...	2.000	+ 39.82	+ 52.42	+ 62.09	+ 72.25	+ 83.25	+ 98.17	2.47
Maltose...	6.021	+111.00	+137.04	+166.11	+176.26	+227.12	+233.36	2.10
Raffinose..	3.713	+ 79.63	+105.20	+131.71	+150.75	+163.77	+188.55	2.37

Average 2.296

It is seen that of the nine sugars galactose shows the greatest and rhamnose the smallest dispersion coefficient, the average value 2.296 being the same as that of sucrose and of glucose.

In 1817 Biot[2] enunciated the law that the specific rotation is in-

[1] *Z. Ver. deut. Zucker-Ind.*, 62, 19 (1912).

[2] *Mem. Acad. Sci.*, 2, 41 (1817).

versely proportional to the square of the wavelength of light, that is, $[\alpha] = A/\lambda^2$, where A is a constant. But it was later found that this law is only approximately correct. Various modified formulas have been proposed for the relationship between specific rotation and wavelength. Lowry and Richards[3] give for sucrose the formula

$$[\alpha] = \frac{21.648}{\lambda^2 - 0.0213}$$

in which the wavelength λ is expressed in microns (one-thousandth of a millimeter).

The results thus calculated have only an approximate value because the specific rotations of the different sugars also vary according to the concentration of solution, the temperature of observation, and the nature of the solvent. Table XXXIX gives the approximate values for the specific rotation of a number of sugars. The effect of concentration and temperature in increasing or lowering the specific rotation is indicated by the direction of the arrow in the respective columns.

TABLE XXXIX

EFFECT OF INCREASE IN CONCENTRATION AND TEMPERATURE UPON SPECIFIC ROTATION OF SUGARS

Sugar	$[\alpha]_D^{20°}$	Increase in Concentration −0+		Increase in Temperature −0+	
Arabinose	+104.5		→		←
Xylose	+ 19.0		→		→
Rhamnose	+ 8.5		?		←
Galactose	+ 80.5		→		←
Glucose	+ 52.5		→		?
Fructose	− 92.5	←		→	
Invert sugar	− 20.0	←		→	
Sucrose	+ 66.5		←		←
Lactose	+ 52.5		?		←
Maltose	+138.5		←		←
Raffinose	+104.5		?		?

EFFECT OF CONCENTRATION UPON SPECIFIC ROTATION OF SUGARS

The effect of varying concentration upon the specific rotation of sugars has been studied by many observers, and the results of their observations have been expressed in the form of equations. The method of deriving these equations, which is due to Biot,[4] is of considerable importance to the sugar chemist and deserves to be briefly considered.

[3] *J. Chem. Soc.*, 125, 2511 (1924).

[4] *Ann. chim. phys.* [3], 10, 385 (1844); 11, 96 (1844); 28, 215 (1850); 36, 257 (1852); 59, 219 (1860).

Concentration Equations. If the specific rotations of a substance for different concentrations are laid off upon a diagram, in which the specific rotations represent the ordinates and the percentages of substance in solution the abscissas, the line which connects the several points will be either a straight line, a section of a parabola, hyperbola, or other curve, or a combination of any two or more of these. Calling the percentage of sugar in solution p, the specific rotation can be represented, according to well-known algebraic equations, as follows:[5]

I	For the straight line	$[\alpha] = a + bp$
II	For the parabola	$[\alpha] = a + bp + cp^2$
III	For the hyperbola	$[\alpha] = a + \frac{bp}{c + p}$

The curve having been plotted and its nature determined, it remains to calculate the values of the constants a, b, and c in the above equations. The method of doing this (the method of least squares) is simple, although the work of calculation is somewhat laborious. The following example is given as an illustration:

From the average results of observations by Tollens, Thomson, Schmitz, Nasini and Villavecchia, the following specific rotations of sucrose were found for different concentrations: 10 per cent +66.56, 20 per cent +66.52, 30 per cent +66.41, 40 per cent +66.27, 50 per cent +66.06. An equation is desired for the specific rotation of sucrose for any concentration within these limits.

By plotting the above observations a curved line is obtained, presumably a parabola. (In calculating the concentration curves for the specific rotation of sugars the hyperbola is but little used.) Substituting the results in the previous equation II for the parabola we obtain the following:

1.	$a + 10\,b + 100\,c = 66.56$
2.	$a + 20\,b + 400\,c = 66.52$
3.	$a + 30\,b + 900\,c = 66.41$
4.	$a + 40\,b + 1600\,c = 66.27$
5.	$a + 50\,b + 2500\,c = 66.06$
Average: I	$a + 30\,b + 1100\,c = 66.364$

[5] It is important to note that Biot based his formulas not upon p, the percentage of optically active substance, but upon e, the percentage of water or other solvent (more frequently designated as q) in which the sugar or other active substance is dissolved. This is the only correct procedure, for, in a solution of several sugars, the polarizing power of each is dependent not upon its individual percentage but upon the percentage of water or other solvent in the solution. Long-persisting errors have resulted in the polariscopic analysis of impure sugar products from not adhering to Biot's original procedure.

From the above equations we obtain by subtraction the following:

6.	(5 − 1)	$40\,b + 2400\,c = -0.50$
7.	(5 − 2)	$30\,b + 2100\,c = -0.46$
8.	(5 − 3)	$20\,b + 1600\,c = -0.35$
9.	(5 − 4)	$10\,b + 900\,c = -0.21$
10.	(4 − 1)	$30\,b + 1500\,c = -0.29$
11.	(4 − 2)	$20\,b + 1200\,c = -0.25$
12.	(4 − 3)	$10\,b + 700\,c = -0.14$
13.	(3 − 1)	$20\,b + 800\,c = -0.15$
14.	(3 − 2)	$10\,b + 500\,c = -0.11$
15.	(2 − 1)	$10\,b + 300\,c = -0.04$
Average: II		$20\,b + 1200\,c = -0.25$

By combining equations 6 to 15 into two series and subtracting, we obtain the following:

III	(7 + 8 + 10 + 12 + 14)	$100\,b + 6400\,c = -1.35$
IV	(6 + 9 + 11 + 13 + 15)	$100\,b + 5600\,c = -1.15$
		$800\,c = -0.20$
		$c = -0.00025$

Substituting the value for c in equation II we obtain $b = 0.0025$, and substituting these values for b and c in equation I we obtain $a = 66.564$. Substituting these values in the original equation for the parabola we obtain:

$$[\alpha]_D^{20} = 66.564 + 0.0025\,p - 0.00025\,p^2$$

The calculated specific rotation of sucrose for various concentrations according to the above equation is as follows: 10 per cent 66.56, 20 per cent 66.51, 30 per cent 66.41, 40 per cent 66.26, 50 per cent 66.06, results which agree well with the average observations taken.

The above equation for the specific rotation of sucrose does not hold, however, for concentrations below 10 per cent or above 50 per cent. Tollens[6] from observations upon 19 solutions ranging from 3.8202 per cent to 69.2144 per cent sucrose calculated the following equations:

For p = 4 to 18 per cent sucrose,

$$[\alpha]_D^{20} = 66.810 - 0.015553\,p - 0.000052462\,p^2$$

For p = 18 to 69 per cent sucrose,

$$[\alpha]_D^{20} = 66.386 + 0.015035\,p - 0.0003986\,p^2$$

According to the above equations the maximum specific rotation of sucrose (66.53) is found at $p = 18.86$ per cent; for concentrations lower than this the specific-rotation again decreases.

[6] *Ber.*, 10, 1403 (1877).

Schmitz[7] from observations upon eight solutions for $p = 5$ to 65 per cent gives the equation:

$$[\alpha]_D^{20} = 66.510 + 0.004508\,p - 0.00028052\,p^2$$

Nasini and Villavecchia[8] for $p = 3$ to 65 give the equation $[\alpha]_D^{20} = 66.438 + 0.010312\,p - 0.00035449\,p^2$. The last-named authorities found, however, for very dilute solutions ($c = 0.335$ g. to 1.2588 g. sucrose per 100 ml.) that the specific rotation of sucrose again increases, and for such dilute solutions give the equation $[\alpha]_D^{20} = 69.962 - 4.86958\,p + 1.86415\,p^2$. The variations noted in the above equations for the specific rotation of sucrose are no doubt partly due to the effect of rotation dispersion, as the result of using light of slightly different wavelength for illumination.

The equations of Tollens and of Nasini and Villavecchia are considered to be the most accurate. The average of the two equations gives probably the most reliable expression for the specific rotation of sucrose.

I $\quad [\alpha]_D^{20} = +66.386 + 0.015035\,p - 0.0003986\,p^2$ (Tollens)

II $\quad [\alpha]_D^{20} = +66.438 + 0.010312\,p - 0.0003545\,p^2$ (Nasini and Villavecchia)

Average: III $\quad [\alpha]_D^{20} = +66.412 + 0.012673\,p - 0.0003766\,p^2$

Landolt[9] by recalculating this combined equation into terms of concentration (grams of sugar per 100 ml.) gives the expression:

IV $\quad [\alpha]_D^{20} = +66.435 + 0.00870\,c - 0.000235\,c^2$ ($c = 0$ to 65)

According to Schönrock, the figure 66.435 in formula IV, giving the specific rotation of sucrose at 0 concentration, is too low. In 1928 he gave the corrected value 66.473,[10] but later changed it to 66.469.[11] This results in the formula

V $\quad [\alpha]_D^{20} = +66.469 + 0.00870\,c - 0.000235\,c^2$ ($c = 0$ to 65)

The specific rotation of sucrose for a concentration of 26 g., weighed in air (26.016 g. in vacuo), in 100 ml., calculated by equation V, is 66.536, which checks with the value calculated from the actual rotation of this solution, 34.620°, accepted by the International

[7] *Ber.*, 10, 1414 (1877).
[8] *Pub. lab. chim. delle gabelle. Rome,* 1891, p. 47.
[9] " Das optische Drehungsvermögen," 2nd ed., p. 420, 1898.
[10] Geiger's " Handbuch der Physik," Vol. 19, p. 705, 1928.
[11] Henning, " Kohlrausch's Praktische Physik," 17th ed., p. 439, 1935.

Commission for Uniform Methods of Sugar Analysis:

$$[\alpha]_D^{20} = \frac{100 \times 34.620}{2 \times 26.016} = 66.536$$

Table XL, which with the exception of column *f* is taken from Landolt, gives a comparison of the specific rotation of sucrose for solutions of different percentage and concentration, according to each of the equations I to IV.

TABLE XL

SPECIFIC ROTATION OF SUCROSE FOR DIFFERENT CONCENTRATIONS

a	*b*	*c*	*d*	*e*	*f*	*g*
			Specific Rotation $[\alpha]_D^{20}$			
Percentage *p*	Density $\frac{20°}{4°}$ (Tollens) *d*	Concentration ($c = p.d.$) (Tollens) *c*	By Formula I Calculated to *p*	By Formula II Calculated to *p*	By Formula III Calculated to *p*	By Formula IV Calculated to *c*
5	1.01786	5.0893	+66.451	+66.480	+66.466	+66.473
10	1.03819	10.3819	66.496	66.506	66.501	66.500
15	1.05926	15.8889	66.522	66.513	66.517	66.514
20	1.08109	21.6218	66.527	66.502	66.515	66.513
25	1.10375	27.5938	66.513	66.474	66.493	66.496
30	1.12721	33.8163	66.479	66.428	66.453	66.460
35	1.15153	40.3036	66.424	66.365	66.394	66.404
40	1.17676	47.0704	66.350	66.283	66.316	66.324
45	1.20288	54.1296	66.256	66.184	66.220	66.217
50	1.22995	61.4975	66.142	66.067	66.104	66.081

If the specific rotation is calculated by formula V, the value for each concentration is 0.034 higher than that shown in column *g*.

Concentration equations, based on *p* or *c*, for the specific rotation of other sugars are given below:

Arabinose[12] (p = 5 to 20 per cent) $[\alpha]_D^{20} = +108.2 - 0.4\,p + 0.014\,p^2$

Xylose[13] (c = 3 to 34 g. per 100 ml.) $[\alpha]_D^{20} = +18.095 + 0.06986\,p$

(c = 34 to 61 g. per 100 ml.) $[\alpha]_D^{20} = +23.089 - 0.1827\,p + 0.00312\,p^2$

Glucose[14] (p = 0 to 100 per cent) $[\alpha]_D^{20} = +52.50 + 0.018796\,p + 0.00051683\,p^2$

[15](c = 6 to 32 g. per 100 ml.) $[\alpha]_{546.1}^{20} = +62.032 + 0.0422\,p + 0.0001897\,p^2$

[12] Von Faber, *Z. angew. Chem.*, 1899, 962.

[13] Schulze and Tollens, *Ann.*, 271, 40 (1892).

[14] Tollens, *Ber.*, 17, 2238 (1884).

[15] Jackson, *Bur. Standards Sci. Paper* 293, p. 633 (1916).

Fructose[16] (p = 1 to 30 per cent) $[\alpha]_D^{20} = -(91.50 + 0.133\,p)$
[17](p = 2.6 to 18.6 per cent) $[\alpha]_D^{25} = -(88.50 + 0.145\,p)$
Invert sugar[18] (p = 9 to 68 per cent) $[\alpha]_D^{20} = -19.447 - 0.06068\,p + 0.000221\,p^2$
Galactose[19] (p = 5 to 35 per cent) $[\alpha]_D^{20} = +79.703 + 0.0785\,p$
Maltose[20] (p = 5 to 35 per cent) $[\alpha]_D^{20} = +138.475 - 0.01837\,p$

The following equations are based on c values:

Glucose[21] (p = 0 to 100 per cent) $[\alpha]_D^{20} = +52.50 + 0.0227\,c + 0.00022\,c^2$
[15](c = 6 to 32 g. per 100 ml.) $[\alpha]_{5461}^{20} = +62.032 + 0.04257\,c$
Fructose[17,22] (c = 2.6 to 20 g. in 100 ml.) $[\alpha]_D^{20} = -(91.33 + 0.164\,c - 0.00086\,c^2)$
Invert sugar[22] (c = 2.6 to 20 g. in 100 ml.) $[\alpha]_D^{20} = -(19.415 + 0.07065\,c - 0.00054\,c^2)$

Effect of Temperature upon Specific Rotation of Sugars

The effect of temperature upon the specific rotation of sugars is no less pronounced than that of concentration; indeed, with a number of sugars such as fructose and galactose, it is the factor which has most to be considered in polarimetric measurements. The change in rotation of a sugar solution due to expansion or concentration in volume through temperature changes must not be confused with changes in specific rotation. In studying the latter phenomenon, either the sugar solutions must be made up to volume at the same temperature at which they are to be examined or else a correction must be made for the changes in volume due to expansion or contraction.

The influence of temperature upon specific rotation is studied in the same way as that of concentration, by laying off the specific rotation for each temperature upon a diagram. The connecting points for the ordinary ranges of atmospheric temperature lie more nearly in a straight line than the points for the concentration curves. For wider ranges of temperature, however, the increase or decrease in

[16] Ost, *Ber.*, **24**, 1636 (1891).
[17] Vosburgh, *J. Am. Chem. Soc.*, **42**, 1696 (1920).
[18] Gubbe, *Ber.*, **18**, 2207 (1885); see Zerban, *J. Am. Chem. Soc.*, **47**, 1104 (1925).
[19] Meissl, *J. prakt. Chem.* [2], **22**, 97 (1880).
[20] Meissl, *J. prakt. Chem.* [2], **25**, 114 (1882).
[21] Browne, *J. Ind. Eng. Chem.*, **2**, 526 (1910).
[22] Zerban, *J. Am. Chem. Soc.*, **47**, 1104 (1925).

specific rotation is found to proceed unequally, and the change must then be expressed by some curve equation.

Effect of Temperature upon the Specific Rotation of Sucrose. The earlier investigators Mitscherlich, Hesse, and Tuchschmid regarded the effect of temperature upon the specific rotation of sucrose as insignificant. Dubrunfaut[23] was the first to recognize the fact that increase of temperature caused a decrease in the value of this constant, the temperature coefficient of the specific rotation of sucrose having been found by him to be 0.000232 per 1° C. increase. Andrews,[24] who reinvestigated the question in 1889, found a decrease of 0.0114 in the specific rotation of sucrose for 1° C. increase. The specific rotation of sucrose for any temperature t is then represented by the equation:

$$[\alpha]_D^t = [\alpha]_D^{20} - 0.0114\,(t - 20)$$

Schönrock[25] in 1896, as a result of observation upon ten sugar solutions, showed that the decrease in specific rotation for 1° C. increase lies between 0.0132 and 0.0151; for temperatures between 12° C. and 25° C. the change is expressed by the equation:

$$[\alpha]_D^t = [\alpha]_D^{20} - 0.0144\,(t - 20)$$

This equation is sometimes written

$$[\alpha]_D^t = [\alpha]_D^{20} - [\alpha]_D^{20}\,0.000217\,(t - 20)$$

in which the temperature coefficient of the specific rotation

$$0.000217 = \frac{0.0144}{[\alpha]_D^{20}} \quad \text{or} \quad \frac{0.0144}{66.5}$$

Later experiments were made by Schönrock[26] at temperatures between 9° C. and 32° C. using light of three different wavelengths: the yellow sodium line 589.3 mμ, the yellow-green mercury line 546.1 mμ, and the blue mercury line 435.9 mμ. These experiments showed that for the German normal sugar solution ($p = 23.704$ per cent) the rotation angle underwent a linear deviation with changes in temperature, this deviation being independent of the wavelength of light employed. It was found, moreover, that the temperature coefficient of the specific rotation decreased with increase in temperature, the value being 0.000242 at 10° C., 0.000184 at 20° C., and 0.000121 at 30° C. for sodium light. This decrease proceeds in a straight line, and the values of the temperature coefficient for any intermediate temperature

[23] *Ann. chim. phys.* [3], 18, 201 (1846).
[24] *Mass. Inst. Tech. Quart.*, May, 1889, p. 367.
[25] *Ber. phys.-techn. Reichsanstalt*, 1896.
[26] *Z. Ver. deut. Zucker-Ind.*, 53, 650 (1903).

can be estimated by taking the proportionate difference. These later values of Schönrock are used by the Physikalisch-Technische Reichsanstalt of Germany.

Effect of Temperature upon the Specific Rotation of Other Sugars. The effect of temperature upon the specific rotations of a number of other sugars is given in Table XLI.

TABLE XLI

Rhamnose[27]	$[\alpha]_D^t = +\ 9.18 - 0.035\,t$ ($t = 6°$ to 20° C.)
Galactose[28] ($p = 10$)	$[\alpha]_D^t = +\ 84.67 - 0.209\,t$ ($t = 10°$ to 30° C.)
Fructose[29] ($p = 9$)	$[\alpha]_D^t = -103.92 + 0.671\,t$ ($t = 13°$ to 40° C.)
Fructose[29] ($p = 23.5$)	$[\alpha]_D^t = -107.65 + 0.692\,t$ ($t = 9°$ to 45° C.)
Invert sugar[30] ($c = 17.21$)	$[\alpha]_D^t = -\ 27.9\ + 0.32\ t$ ($t = 5°$ to 35° C.)
Lactose[31] ($c = 5$)	$[\alpha]_D^t = +\ 52.42 + 0.072(20 - t)$; ($t = 15°$ to 28° C.)
Maltose[32] ($p = 10$)	$[\alpha]_D^t = +\ 140.19 - 0.095\,t$ ($t = 15°$ to 35° C.)

While a linear equation is sufficiently exact for narrow ranges of temperature, the change in specific rotation for wider differences of temperature must usually be expressed by an equation of the order:

$$[\alpha]_D^t = [\alpha]_D^0 + at + bt^2$$

or

$$[\alpha]_D^t = [\alpha]_D^{20} + a(t - 20) + b(t - 20)^2$$

Gernez,[33] for example, gives for rhamnose the equation

$$[\alpha]_D^t = 9.22 - 0.03642\,t + 0.0000123\,t^2$$

and Gubbe[34] gives for invert sugar the following equations:

For $t = 0°$ to 30° C., $[\alpha]_D^t = [\alpha]_D^{20} + 0.3041\ (t - 20) + 0.00165\ (t - 20)^2$

For $t = 20°$ to 100° C., $[\alpha]_D^t = [\alpha]_D^{20} + 0.3246\ (t - 20) - 0.00021\ (t - 20)^2$

The temperature coefficient may also vary with the sugar concentration. For fructose concentrations between 5 and 10 g. per 100 ml. Vosburgh[35] has found

$$[\alpha]_D^t = [\alpha]_D^{20} + (0.566 + 0.0028\,c)\ (t - 20);\ (t = 15 \text{ to } 37° \text{ C.})$$

For the same concentrations of invert sugar, Zerban[36] gives

$$[\alpha]_D^t = [\alpha]_D^{20} + (0.283 + 0.0014\,c)\ (t - 20);\ (t = 15 \text{ to } 35° \text{ C.})$$

[27] Schnelle and Tollens, *Ann.*, **271**, 62 (1892).
[28] Meissl, *J. prakt. Chem.* [2], **22**, 97 (1880).
[29] Hönig and Jesser, *Z. Ver. deut. Zucker-Ind.*, **38**, 1028 (1888).
[30] Tuchschmid, *J. prakt. Chem.* [2], **2**, 235 (1870).
[31] Bacharach, *Analyst*, **48**, 521 (1923).
[32] Meissl, *J. prakt. Chem.* [2], **25**, 114 (1882).
[33] *Compt. rend.*, **121**, 1150 (1895).
[34] *Ber.*, **18**, 2207 (1885).
[35] *J. Am. Chem. Soc.*, **42**, 1696 (1920).
[36] *J. Am. Chem. Soc.*, **47**, 1104 (1925).

Sucrose and the different sugars mentioned in Table XLI all show a decrease in specific rotation with increase in temperature. Of other sugars which exhibit this property in marked degree, arabinose should be mentioned. Tanret[37] found for *l*-arabinose $[\alpha]_D^{12} = +105.54$ and $[\alpha]_D^{55} = +88.61$, or an average decrease of 0.394 for 1° C. increase in temperature, which is greater than that for any other sugar except fructose.

Xylose presents an exception to the rule just noted, Schulze and Tollens[38] having observed for temperatures above 20° C. an increase in specific rotation, as in the following example ($p = 10.0829$).

t	$[\alpha]_D$ *d*-Xylose
15°	+18.898
20	18.909
25	19.248
30	19.628

Glucose also seems to present an exception to the rule of diminished rotation with increase in temperature. Observations by Dubrunfaut, Mategczek, and others show that the specific rotation of *d*-glucose undergoes no perceptible change between 0° and 100° C.

Equations giving the combined influence of concentration and temperature upon specific rotation have been worked out for many sugars. The following examples are given:

Galactose[39] $[\alpha]_D^t = +83.883 + 0.0785\,p - 0.209\,t$ (Meissl)[40]

Fructose $[\alpha]_D^t = -[101.38 - 0.56\,t + 0.108\,(c - 10)]$ (Jungfleisch and Grimbert)[41]

Fructose $[\alpha]_D^t = -88.13 - 0.2583\,p + 0.6714\,(t - 20°)$ (Hönig and Jesser)[42]

Sorbose $[\alpha]_D^{20} = -[42.65 + 0.047\,p + 0.00007\,p^2 - (t - 20)0.02]$ (Tollens and Smith)[43]

Maltose $[\alpha]_D^{20} = +140.375 - 0.01837\,p - 0.095\,t$ (Meissl)[44]

[37] *Bull. soc. chim.* [3], 15, 195 (1896).

[38] *Ann.*, 271, 40 (1892).

[39] Tanret (*Bull. soc. chim.* [3], 15, 195), gives the change in specific rotation of galactose for 1° C. increase between 13° and 20° —0.39, between 20° and 25° —0.226, and between 25° and 30° —0.180, a falling off in the temperature coefficient with increase in temperature similar to the one noted by Schönrock with sucrose. Mackenzie and Ghosh (*Proc. Royal Soc. Edinburgh*, 35, 22 [1915]), however, report a coefficient of —0.23 at 12.5°, and —0.34 at 18°.

[40] *J. prakt. Chem.* [2], 22, 97 (1880).

[41] *Compt. rend.*, 107, 390 (1888).

[42] *Z. Ver. deut. Zucker-Ind.*, 38, 1028 (1888).

[43] *Ber.*, 33, 1289 (1900).

[44] *J. prakt. Chem.* [2], 25, 114 (1882).

Effect of Solvent upon the Specific Rotation of Sugars

The constants of specific rotation for sugars are all expressed for aqueous solutions. It sometimes happens, however, that solutions of sugar in other solvents, such as alcohol, have to be examined; then the changes in specific rotation due to the character of solvent must be taken into account.

In the case of sucrose, Tollens[45] found the following values for $[\alpha]_D^{20}$ with different solvents for a 10 per cent solution:

In water	+ 66.667
In 1 part water + 3 parts ethyl alcohol	+ 66.827
In 1 part water + 3 parts methyl alcohol	+ 68.628
In 1 part water + 3 parts acetone	+ 67.396

Methyl alcohol and acetone are thus seen to raise the specific rotation of sucrose perceptibly, but ethyl alcohol only slightly. Claassen[46] also found for 80 per cent alcohol a slight increase in the specific rotation of sucrose; the differences (0.1 to 0.15), however, are not sufficient to affect seriously the analytical results in such operations as the alcoholic extraction of sugar beet or cane pulp.

The specific rotation of other sugars dissolved in alcohol may be seen from Table XLII, giving results obtained by Hudson and Yanovsky.[47]

TABLE XLII

Sugar	Solvent	Specific Rotation at 20° C.
Glucose	80% alcohol	+ 59.0
Glucose	Absolute alcohol	+ 70.45
Fructose	80% alcohol	− 68.6
Fructose	95% alcohol	− 52.5
Fructose	Methyl alcohol	− 61.4
Galactose	60% alcohol	+ 72.8
Galactose	80% alcohol	+ 73.1
Mannose	80% alcohol	+ 25.7
Mannose	Methyl alcohol	+ 30.1
Lactose	40% alcohol	+ 55.3
Maltose	60% alcohol	+128.1
Arabinose	80% alcohol	− 81.7
Xylose	80% alcohol	+ 32.1

In all these sugars except lactose, ethyl or methyl alcohol produced a marked change in the specific rotation, and when these sugars are present the influence of alcohol must be taken into account.

[45] *Ber.*, **13**, 2287 (1880).
[46] *Z. Ver. deut. Zucker-Ind.*, **40**, 392 (1890).
[47] *J. Am. Chem. Soc.*, **39**, 1013 (1917).

Borntraeger[48] found for 37.6 g. invert sugar in 100 ml. aqueous solution a rotation of —49.2 at 20° C.; when the solution was made up with 10.45 ml. alcohol, the rotation decreased to —43.9, and with 20.60 ml. alcohol to —38.3. According to Horsin-Déon[49] invert sugar in absolute alcohol is perfectly inactive and becomes levorotatory only upon the addition of water. This statement, however, is probably incorrect because glucose in absolute alcohol shows $[\alpha]_D^{20} = +70.45$ while that of fructose in the same solvent is evidently lower than —52.5. It should be noted that the rotation of alcoholic invert-sugar solutions is much more sensitive to changes in temperature than that of aqueous solutions.

With a number of sugars the specific rotations in aqueous and alcoholic solutions are almost the reverse of each other. The $[\alpha]_D$ of rhamnose,[50] for example, in water is +9.43 and in alcohol —9.0. The $[\alpha]_D$ of sorbose[51] in water is —42.5 and in 85 per cent alcohol +41.8. The effect of pyridine and formic acid upon the specific rotations of several sugars is shown on p. 288.

Without giving detailed results of experiments upon all the various sugars it may be said that the effect of solvent upon specific rotation is too great to be disregarded; wherever possible the polarimetric examination of sugars for purpose of analysis should be made in aqueous solution.

Effect of Accompanying Substances upon Specific Rotation of Sugars

Another factor of importance, especially in the polarimetric examination of impure sugar solutions, is the effect which bases, acids, salts, and other substances exert upon the specific rotation of the sugars present. A very large amount of investigation has been done upon this subject, and for complete details reference must be made to the original articles. Only brief mention will be made of the effects of a few substances upon the rotation of the more important sugars.

The changes which foreign optically inactive substances may exert upon the rotation of sugars may be either chemical or physical. The hydroxides of the alkalies and alkaline earths, and all salts of alkaline reaction in general, cause a decrease in the specific rotation of most reducing sugars. Such changes in rotation are largely chemical, being

[48] *Z. angew. Chem.*, **1889**, 507.
[49] *J. fabr. sucre*, **20**, 37 (1879).
[50] Raýman and Kruis, *Bull. soc. chim.* [2], **48**, 632 (1887).
[51] Adriani, *Rec. trav. chim.*, **19**, 184 (1900).

due either to a rearrangement of the sugar molecule or to the formation of alkali-sugar compounds of lower specific rotation. The effect of acids and acid salts upon the rotation of sucrose by inversion is another example of purely chemical change. The avoidance of such chemical changes is imperative in accurate polarimetric work, and to prevent these the solutions of sugar under examination should, so far as possible, be neutral in reaction.

The influence of neutral salts upon the specific rotation of sugars, on the other hand, is largely physical, since the chemical properties of the dissolved sugars are not appreciably affected; the same is also true of the influence of acids upon the specific rotation of sugars which do not undergo inversion.

Influence of Mineral Impurities upon the Rotation of Sucrose. The chlorides, nitrates, sulfates, phosphates, acetates, and citrates of the alkalies, the chlorides of the alkaline earths, magnesium sulfate, and many other salts all produce a decrease in the specific rotation of sucrose, this decrease being generally greater with increased amount and smaller molecular weight of salt.

The hydroxides of the alkalies and alkaline earths and the carbonates of the alkalies also lower the specific rotation of sucrose. The influence of these substances, which is of especial importance technically, in view of the alkalinity of various sugar-house products, has been widely studied, the results often being expressed in parts of sugar whose rotation is obscured by one part of alkali. Pellet, for example, gives the following results:

Substance	Concentration of Sucrose Solution	
	5.4 g. 100 ml.	17.3 g. 100 ml.
	grams sucrose	grams sucrose
1 g. caustic potash obscures rotation of.....	0.170	0.500
1 g. caustic soda obscures rotation of.....	0.140	0.450
1 g. potassium carbonate obscures rotation of..	0.044	0.065
1 g. sodium carbonate obscures rotation of...	0.040	0.132
1 g. calcium oxide obscures rotation of.....	0.7	1.0
1 g. barium oxide obscures rotation of.....	0.190	0.430

Strontium oxide also diminishes the specific rotation of sucrose. This lowering effect of alkalies upon the specific rotation of sucrose is largely due to the formation of soluble saccharates of lower specific rotations.

Smoleński and Kozlowski[52] have shown that the rotation of alkaline sucrose solutions is a function of their *p*H and depends on the proportions of dissociated and undissociated sucrose molecules. Considering sucrose as a dibasic acid, the first dissociation constant is about 3×10^{-13}, and the second dissociation constant about 3×10^{-14}. The molecular rotation M (specific rotation $\times$ molecular weight $\times$ 0.01) of the total sucrose in alkaline solution is given by the formula

$$M = \alpha_1 \gamma_1 + \alpha_2 \gamma_2 + \alpha (1 - \gamma_1 - \gamma_2)$$

where γ_1 is the concentration of the sucrose ionized by the first dissociation, γ_2 that of the sucrose ionized by the second dissociation, and $(1 - \gamma_1 - \gamma_2)$ that of the undissociated sucrose; α_1, α_2, and α are the corresponding molecular rotations.

The molecular rotation of the undissociated molecules was found to be about 22.6 ($[\alpha]_D^{20} = 66.1$). $\alpha_1 = \dfrac{21.66 - 0.525\,\alpha}{0.475} = 20.6$ ($[\alpha]_D^{20} = 60.2$); $\alpha_2 = \alpha - 4.24 = 18.4$ ($[\alpha]_D^{20} = 53.8$).

The effect of alkali on the rotation of sucrose can be partly eliminated by neutralization with acetic acid. The original specific rotation is not entirely restored, however, since the soluble acetates themselves lower the specific rotation of sucrose to a slight extent.

The probable effect of a mixture of salts upon the polarization of sucrose — such for example as occurs in beet molasses, which contains about 50 per cent of sucrose and 10 per cent of soluble salts (mostly of potassium) — may be judged from the examples taken from experiments by Bodenbender and Steffens[53] and shown in Table XLIII.

The fourfold concentration is seen to depress the difference in rotation about ten times, so that an apparent loss of sucrose may seem to take place in the evaporation of sugar solutions rich in mineral salts when such solutions are examined by the polariscope before and after evaporation.

Later investigations by Farnsteiner,[54] by Kunst,[55] and by Landt[56] have shown that the rotation depression increases inversely with the hydration of the ions. Thus the depression effect of equimolecular concentrations of cations at constant sucrose concentration is in the following order:

$$\mathrm{Na} > \mathrm{K} > \mathrm{Li}$$

[52] *Bull. assoc. chim. sucr. dist.*, 53, 837 (1936).
[53] *Z. Ver. deut. Zucker-Ind.*, 31, 808 (1881).
[54] *Ber.*, 23, 3570 (1890).
[55] *Arch. Suikerind.*, 41, I, 657 (1933).
[56] *Deut. Zuckerind.*, 60, 902 (1935); 61, 377 (1936).

for anions the sequence is as follows:

$$I > Br > Cl > F$$

With bivalent ions the relationships are more complicated. At small salt concentrations the depressing effect is about the same for $MgCl_2$, $CaCl_2$, and $SrCl_2$. At small concentration $BaCl_2$ gives only a slight depression; as its concentration increases, the depression reaches a

TABLE XLIII

Salt	Sucrose, Parts	Salt, Parts	Water, Parts	Polarization, Sugar Degrees	Difference
Potassium chloride	5	1	94	4.987	0.013
	10	2	88	9.856	0.144
	20	4	76	19.869	0.131
Sodium chloride	5	1	94	4.969	0.031
	10	2	88	9.853	0.147
	20	4	76	19.586	0.414
Barium chloride	5	1	94	4.952	0.048
	10	2	88	9.944	0.056
	20	4	76	19.402	0.598
Magnesium sulfate	5	1	94	4.995	0.005
	10	2	88	9.890	0.110
	20	4	76	19.880	0.120
Sodium phosphate	5	1	94	4.958	0.042
	10	2	88	9.933	0.067
	20	4	76	19.689	0.311
Potassium carbonate	5	1	94	4.927	0.073
	10	2	88	9.730	0.270
	20	4	76	19.300	0.700
Sodium carbonate	5	1	94	4.910	0.090
	10	2	88	9.711	0.289
	20	4	76	19.173	0.827

maximum and then decreases again. Similar maxima are shown by $CaCl_2$ and $SrCl_2$. If only the second portion of the curves, beyond the maxima, is considered, the depressing effect of the salts is in the following order:

$$CaCl_2 > SrCl_2 > BaCl_2$$

which is analogous to the sequence of the univalent cations. At very low concentrations $MgCl_2$ has a smaller effect than $BaCl_2$, in accordance with theory.

Although the primary cause of the depression is a dehydrating effect of the ions on the sucrose-water complex, the ions also affect the sugar molecule itself, either by complex formation in the case of large ions, or by deformation of the sucrose molecule through electrostatic forces in the case of the smaller, highly hydrated ions.

Working with the saccharimeter, Jackson and Gillis[57] found that the polarization P, in degrees S., of a normal weight solution of sucrose, to which m grams of various salts are added, may be expressed by these equations:

Sodium chloride	$P = 100 - 0.265\,m$
Potassium oxalate	$P = 100 - 0.234\,m$
Calcium chloride	$P = 100 - 0.339\,m$
Ammonium chloride	$P = 100 - 0.169\,m$

The depressing effect is proportional not only to the salt concentration but also to the sucrose concentration, as expressed by the formula of Brown:[58]

$$P = P' - kP'm$$

where P' is the polarization of the sucrose solution without added salt, and P that of the same solution with m grams of salt added, in a total volume of 100 ml. The constant k has the following value for various salts:

NaCl	0.00246
K_2SO_4	0.00199
$Na_2SO_4 \cdot 10\ H_2O$	0.00205
$Na_2HPO_4 \cdot 12\ H_2O$	0.00305

The value 0.00246 for sodium chloride, or 0.246 on the basis of 100 polarization, checks quite well with the figure found by Jackson and Gillis.

The effect which the various salts used for clarifying impure sugar solutions for optical analysis exercise upon the specific rotation of sucrose and other sugars is also of great importance. Lead subacetate is the salt most used for this purpose; its effect upon the rotation of sucrose is considered elsewhere (p. 323).

Influence of Mineral Impurities upon the Rotation of Reducing Sugars. The action of salts of alkaline reaction in depressing the rotation of reducing sugars has already been mentioned. In saccharimetric analysis the influence of lead subacetate, as a clarifying agent, upon the rotations of fructose and invert sugar, is of great importance.

As was first observed by Gill[59] in 1871 when solutions containing invert sugar are treated with lead-subacetate solution in excess, the formation of soluble lead fructosate of low specific rotation is so pronounced that the rotatory power of fructose sinks below that of glucose and the invert sugar becomes dextrorotatory. Similar observations have been made by Pellet, Bittmann, Koydl, Borntraeger, and many

[57] *Bur. Standards Sci. Paper* 375, 1920.
[58] *Ind. Eng. Chem.*, 17, 39 (1925).
[59] *Z. Ver. deut. Zucker-Ind.*, 21, 257 (1871).

others. In the following experiments by Bittmann[60] 50 ml. of invert-sugar solution was treated with 50 ml. of a mixture of water and lead subacetate in different proportions.

Water	Lead Subacetate Solution	Polarization
ml.	ml.	
50	0	−2.3
40	10	−1.0
30	20	+3.7
10	40	+7.5

Jackson and Gillis[61] found that each gram of dry lead subacetate, added to the inverted half-normal-weight solution of sucrose, lowers the levorotation 0.715° S., or 1.43° S. on the normal-weight basis.

The influence of neutral salts upon the specific rotation of reducing sugars is variable. Some salts produce an increase, others a decrease and some no change whatever in rotation; no general rule can be given. As an example, a study by Murschhauser,[62] regarding the effect of a large number of salts on the specific rotation of glucose, may be cited.

Of particular importance in this connection is the influence of different neutral salts upon the rotation of invert sugar; the occurrence of such salts in molasses and other low-grade sugar-house products may increase the levorotation of the invert sugar several degrees, with the result that erroneous conclusions are sometimes drawn from the polariscopic examination of such products.

Jackson and Gillis give the following formulas for the effect of various salts on the rotation of invert sugar, in degrees S. at 20° C., for the inverted half-normal weight of sucrose, but referred to the normal-weight basis; m is grams salt in 100 ml. solution:

NaCl	$-32.00 - 0.540\,m$
KCl	$-32.00 - 0\,486\,m$
NH_4Cl	$-32.00 - 0.563\,m$
$CaCl_2$	$-32.00 - 0.710\,m$
$K_2C_2O_4$	$-32.00 - 0.510\,m$
NH_4NO_3	$-32.00 - 0.399\,m$
$Na_2HPO_4 \cdot 12H_2O$	$-32.00 - 0.161\,m$
$NaC_2H_3O_2 \cdot 3H_2O$	$-32.00 - 0.189\,m$
$Pb(C_2H_3O_2)_2$	$-32.00 - 0.020\,m$

[60] *Z. Ver. deut. Zucker-Ind.*, 30, 875 (1880).
[61] *Bur. Standards Sci. Paper* 375, p. 158, 1920.
[62] *Biochem. Z.*, 136, 66 (1923).

According to Tomoda and Taguchi,[63] sodium bisulfite lowers the polarizing power of glucose, arabinose, galactose, and lactose markedly, and that of maltose slightly, but has practically no effect on that of fructose, mannose, sucrose, raffinose, or dextrin. This principle is made use of by Tomoda and Taguchi in a method for analyzing mixtures of glucose and fructose, like honey, or of glucose and maltose. The error of the method is as high as 6.5 per cent, and it is therefore of little practical value.

Influence of Acids upon the Specific Rotation of Sugars. The presence of free mineral acids exerts a very pronounced influence upon the specific rotation of certain sugars. This influence is slight for glucose, except at very high acid concentration,[64] but is most pronounced with fructose and hence also with invert sugar. O'Sullivan, for example, found for invert sugar, prepared by inverting sucrose with invertase, $[\alpha]_D^{15} = -24.5$, and for invert sugar, prepared by inverting sucrose with sulfuric acid in the cold, $[\alpha]_D^{15} = -27.7$, an increase of 3.2, which if referred entirely to fructose would mean an increase of 6.4 in the specific rotation of that sugar. The increase in rotation increases with the amount of acid, as is seen from the following results by Hammerschmidt[65] which Browne has calculated to the $[\alpha]_D^{20}$ of invert sugar and fructose. The results were obtained by inverting a half-normal weight of sucrose with varying amounts of concentrated hydrochloric acid and then completing the volume to 100 ml.

TABLE XLIV

INFLUENCE OF VARYING QUANTITIES OF HYDROCHLORIC ACID UPON THE ROTATION OF INVERT SUGAR AND FRUCTOSE

Volume of HCl Added	Observed Saccharimeter Reading, 20° C. (13.6842 g. invert sugar to 100 ml.)	Calculated $[\alpha]_D^{20}$	
		Invert Sugar	Fructose
ml.	° V.		
0	.....	−20.00	−92.50
5	−16.50	−20.89	−94.28
10	−17.06	−21.60	−95.70
15	−17.58	−22.26	−97.02
20	−18.02	−22.82	−98.14

According to Jackson and Gillis[66] the effect of hydrochloric acid and

[63] *J. Soc. Chem. Ind., Japan*, 33, Suppl., 434 (1930).
[64] Zechmeister, *Z. physik. Chem.*, 103, 316 (1922).
[65] *Z. Ver. deut. Zucker-Ind.*, 40, 465 (1890); 41, 157 (1891).
[66] *Loc. cit.*

of phosphoric acid on the saccharimetric reading of invert sugar, in degrees S. at 20° C., may be expressed by the formulas

$$\begin{aligned} HCl \quad & -32.00 - 0.5407\,m \\ H_3PO_4 \quad & -32.00 - 0.0776\,m \end{aligned}$$

The influence of the change in specific rotation of fructose upon the determination of sucrose by the methods of acid inversion is discussed on p. 417. The action of organic acids upon the rotation of fructose and invert sugar is much less pronounced than that of mineral acids, and can usually be disregarded in polariscopic analysis. Acetic acid actually causes a lowering in the levorotation of invert sugar ($-32.00 + 0.0823\,m$).

Influence of Foreign Optically Active Substances upon the Specific Rotation of Sugars. The effect of other optically active ingredients upon the rotation of a sugar is of importance especially in determining the polarizing power of several sugars in solution or of mixtures of sugars with organic non-sugars which are optically active. The difficulties in conducting studies of this kind seem to have deterred investigation somewhat; the earlier studies upon the polarizing power of sugar mixtures showed no change in the rotation of the individual sugars.

The polarizing power of solutions of sucrose and glucose in different proportions was found by Hammerschmidt[67] to agree with the sum of the values calculated by the concentration formulas of Tollens (p. 269) within experimental limits of error. Similar results were also obtained by Creydt[68] with cane sugar and raffinose. Results by Browne[69] upon the polarization of mixtures of glucose and fructose, glucose and galactose, fructose and galactose, fructose and arabinose, arabinose and xylose also show that it is safe to assume in analytical work that the specific rotation of these sugars is not perceptibly affected by other sugars in solution.

Later it was found, however, that, although specific rotation is an additive property, the specific rotation of mixed sugars is not the sum of their specific rotations at their partial concentrations, but the sum of the specific rotations which each sugar would have if it were present alone at a concentration equal to the total sugar concentration. In other words, the determining factor is not the partial sugar concentrations, but rather the water concentration, as pointed

[67] "Das spezifische Drehungsvermögen von Gemengen optisch activer Substanzen," Dissertation, Rostock University, 1889.

[68] *Z. Ver. deut. Zucker-Ind.*, **37**, 153 (1887).

[69] *J. Am. Chem. Soc.*, **28**, 339 (1906).

out by Browne,[70] who confirmed Vosburgh's work on this subject. Vosburgh[71] proved this rule to hold for mixtures of glucose and fructose, and of glucose and sucrose; for mixtures of sucrose and fructose the calculated values were slightly higher than the observed.

Mutarotation

A phenomenon observed in the polarization of all optically active reducing sugars is that of mutarotation (formerly called birotation or multirotation). The polarizing power of such sugars undergoes after solution at first a rapid change which slowly becomes more gradual until after a few hours the polariscope reading remains constant. This phenomenon was first observed upon glucose in 1846, by Dubrunfaut,[72] and the fact that the initial rotation of this sugar was about twice the constant value caused the introduction of the name birotation. The relation 2 : 1 was found, however, to be different for other sugars; Wheeler and Tollens,[73] for example, found the ratio for xylose to be about 4.5 : 1 and accordingly suggested the name multirotation. This term, however, has given place to the more expressive word mutarotation (Latin *mutare* = to change) introduced by Lowry[74] in 1899.

The effect of mutarotation upon the rotatory power of sugars is shown in Table XLV, in which results are quoted from the work of Tollens and his coworkers, giving the specific rotation of a number

TABLE XLV

Mutarotation of Different Sugars

Sugar	Grams per 100 ml.	$[\alpha]_D^{20}$ Initial		$[\alpha]_D^{20}$ Constant		Difference
			min.		hours	
l-Arabinose	9.73	+156.7	6.5	+104.6	1.5	−52.1
d-Xylose	10.235	+ 85.9	5.	+ 18.6	2.0	−67.3
d-Glucose	9.097	+105.2	5.5	+ 52.5	4.5	−52.7
d-Galactose	10.000	+117.4	7.	+ 80.3	4.5	−37.1
d-Fructose	10.000	−104.0	6.	− 92.3	0.5	−11.7
Rhamnose	10.000	− 5.0	5.5	+ 9.4	1.0	+14.4
Fucose	6.916	−111.8	11.	− 77.0	2.0	−34.8
Lactose	4.841	+ 87.3	8.	+ 55.3	10.0	−32.0
Maltose	9.2	+118.8	6.	+136.8	6.5	+17.0

[70] *Louisiana Planter*, 67, 44 (1921); see also note on p. 266.
[71] *J. Am. Chem. Soc.*, 43, 219 (1921).
[72] *Compt. rend.*, 23, 38 (1846).
[73] *Ann.*, 254, 312 (1889).
[74] *J. Chem. Soc.*, 75, 212 (1899).

of sugars directly after solution and after standing until no further change was noted. The time after solution is given after each value for $[\alpha]_D^{20}$.

It is noted that for rhamnose there is a decrease in rotation from —5.0 to 0 and then an increase from 0 to +9.4. Maltose also differs from the other sugars in showing less rotation at time of solution than after standing.

Effect of Temperature on Mutarotation. The speed of mutarotation is influenced by a large number of factors. It is accelerated by increase in temperature, the change proceeding very slowly at 0° C., and almost instantly at 100° C. Dilute sugar solutions show approximately the same velocity of change for all concentrations. Highly concentrated solutions, however, do not always give the true end rotation; such solutions must first be diluted and then allowed to stand for the change in rotation to be completed. This fact must be borne in mind in the polariscopic examination of concentrated sugar solutions, such, for example, as liquid honey; otherwise a considerable error may be introduced in the work of analysis.

Velocity of Mutarotation. The velocity of the change from initial to constant rotation is different for different sugars, and also varies according to temperature, solvent, and other conditions. Urech[75] was the first to show that the speed of mutarotation followed the unimolecular law which is the same as that noted by Wilhelmy in the acid inversion of sucrose and is expressed by the following general formula:

$$\frac{dx}{dt} = k(a - x)$$

in which k is the coefficient of velocity, a the total change between the beginning and end point, and x the change at the end of any time t. The above equation by integration gives

$$k = \frac{1}{t} \log \frac{a}{a - x}$$

Owing to the impossibility of measuring the specific rotation of a sugar at the exact moment of solution, the velocity of mutarotation is generally determined by the modified formula

$$k = \frac{1}{t_2 - t_1} \log \left(\frac{\beta_1 - \phi}{\beta_2 - \phi} \right)$$

in which β_1 and β_2 are the rotations at the end of the corresponding times t_1 and t_2, and ϕ the constant end rotation.

[75] *Ber.*, 16, 2270 (1883); 17, 1547 (1884); 18, 3059 (1885).

The method of calculation is shown by the following example, taken from the work of Levy.[76]

TABLE XLVI

VELOCITY OF MUTAROTATION FOR A GLUCOSE SOLUTION

Per cent, $C_6H_{12}O_6$ = 3.502. $d_{4°}^{20°}$ = 1.0114. Temperature = 20.5° to 20.9° C.

Time after Solution	Angular Rotation (8-dm. tube)	$t_2 - t_1$	Temperature	$k = \frac{1}{t_2 - t_1} \log_{10} \left(\frac{\beta_1 - \phi}{\beta_2 - \phi}\right)$
t_1 = 25 min.	β_1 = 27.865°	0	20.9° C.	.....
t_2 = 30 min.	β_2 = 27.060	5	20.9	0.00649
t_2 = 35 min.	β_2 = 26.159	10	20.9	0.00719
t_2 = 40 min.	β_2 = 25.637	15	20.8	0.00644
t_2 = 45 min.	β_2 = 24.927	20	20.7	0.00662
t_2 = 50 min.	β_2 = 24.369	25	20.6	0.00652
t_2 = 55 min.	β_2 = 23.895	30	20.5	0.00636
t_2 = 60 min.	β_2 = 23.166	35	20.5	0.00677
t_2 = 65 min.	β_2 = 22.797	40	20.5	0.00656
t_2 = 70 min.	β_2 = 22.171	45	20.5	0.00687
t_2 = 75 min.	β_2 = 21.837	50	20.5	0.00674
t_2 = 80 min.	β_2 = 21.470	55	20.5	0.00671
t_2 = 85 min.	β_2 = 21.088	60	20.5	0.00675
24 hours	ϕ = 16.692	..	Average	0.00662

The unimolecular velocity constants found by Hudson and Yanovsky[77] for a number of sugars at 20° C. are shown in Table XLVII.

TABLE XLVII

Sugar	Velocity Constant	Sugar	Velocity Constant
Fructose	0.082	α-Glucoheptose	0.0122
Lyxose	0.065	Galactose	0.0102
Rhamnose	0.039	Melibiose	0.0088
Arabinose	0.031	Maltose	0.0072
Fucose	0.022	Glucose	0.0066
Xylose	0.021	Cellobiose	0.0047
Mannose	0.019	Lactose	0.0047

It is seen that the change to constant rotation is most rapid for fructose, and slowest for lactose and cellobiose.

It was later found that the mutarotation of some sugars, notably

[76] *Z. physik. Chem.*, 17, 301 (1895).
[77] *J. Am. Chem. Soc.*, 39, 1013 (1917).

galactose, arabinose, ribose, and sorbose, is not a unimolecular reaction, but more complex. If the velocity coefficient is calculated according to the method outlined above, it shows a rapid change in value at the beginning but after a certain time becomes constant. This constant value may be used to correct the readings observed during the period of rapid change, and the velocity coefficient for the period of rapid change is then calculated in the usual manner from the corrected readings. In this way Isbell and Pigman[78] have established the velocity coefficient for the slow rotation change m_1, and that for the initial rapid change m_2, obtaining the following values at 20° C.

	m_1	m_2
α-*d*-Galactose	0.00803	0.0790
α-*l*-Arabinose	0.0300	0.138
l-Ribose	0.0492	0.231
l-Sorbose	0.040	0.250

The rapid mutarotation constant m_2 of *d*-galactose is of about the same order of magnitude as the mutarotation constant of fructose, and that of the other sugars listed is even considerably higher. The significance of this fact is discussed on p. 292.

Effect of Acids, Bases, and Salts on Mutarotation. The action of acids, bases, and salts upon the velocity of mutarotation has been a subject of considerable study. It has been found that mutarotation takes place only in amphoteric solvents, that is, in the presence of both an acid and a base in the wider sense of proton donor and acceptor, which may be either an ion or a molecule. Water itself is such an amphoteric solvent, and it also acts as a proton donor or acceptor in aqueous solutions of acids or bases. Pure dry pyridine, having only basic properties, or pure dry cresol, having only acid properties, does not catalyze mutarotation, but a mixture of the two is an efficient catalyst.

Acids accelerate mutarotation according to their degree of dissociation, or electric conductivity, preserving approximately the same order as that noted in the inversion of sucrose. Levy,[79] for example, gives the constants shown in Table XLVIII for the speed of mutarotation of glucose in presence of different acids ($_1^1{}_0$ normal) and the relative acceleration of each acid in terms of hydrochloric acid = 100.

Brönsted and Guggenheim[80] have shown that the logarithm of the velocity constant of mutarotation k is an approximately straight-line

[78] *Bur. Standards J. Research*, **18**, 141 (1937); **19**, 443 (1937).
[79] *Z. physik. Chem.*, 17, 301 (1895).
[80] *J. Am. Chem. Soc.*, **49**, 2554 (1927).

TABLE XLVIII

Acceleration of Different Acids upon Mutarotation

In Presence of	Velocity Constant of Mutarotation	Temperature	Relative Acceleration
Water	0.00610	20.1° C.	.....
Water	0.00637	20.25	.....
	.....	.....	.....
Hydrochloric acid	0.02300	20.25	100.00
Nitric acid	0.02283	20.1	98.99
Trichloracetic acid	0.02325	20.25	96.67
Sulfuric acid	0.01886	20.0	71.95
Dichloracetic acid	0.01670	20.2	62.41
Monochloracetic acid	0.01004	20.25	17.25
Acetic acid	0.00716	20.2	4.70
Propionic acid	0.00636	19.8	1.63

function of the logarithm of the dissociation constant K of acids, according to the formula

$$\log k = c + xK$$

where c is an arbitrary constant, and x, denoting the slope of the curve, is about 0.2.

Alkalies are much more effective catalysts of mutarotation than acids, and the slope of the curve correlating the velocity coefficient with the dissociation constant is much steeper than in the case of acids, the value of the constant x in the formula above being about 0.4. Schulze and Tollens,[81] using 0.1 per cent ammonia, obtained the normal constant rotation with arabinose, xylose, rhamnose, galactose, glucose, fructose, and lactose within 9 minutes; $N/200$ alkali (KOH) gives the end rotation of glucose almost instantly. The use of much stronger alkali, however, induces chemical change with a decrease of the rotation below the normal value. Trey,[82] for example, using 0.2 g. sodium hydroxide per 100 ml., obtained as the $[\alpha]_D$ for glucose after 15 minutes +52.7 (normal), after 24 hours +36.7, after 48 hours +26.0, after 34 days +15.1, and after 65 days —0.4.

The velocity constant of mutarotation has a minimum value at about pH 4.6, and increases as the pH is either lowered or raised. The effect on the rapid type of mutarotation is much more pronounced than on the slow type. At pH 1.05 the velocity constant for fructose is 12 times that at pH 4.6, but for d-glucose it is only 3.57 times

[81] *Ann.*, **271**, 49 (1892).
[82] *Z. physik. Chem.*, **22**, 439 (1897).

that at *p*H 4.6. Similarly, at *p*H 6.91 the constant for fructose is 2.45 times that at *p*H 4.6, but for glucose it is only 1.07 times that at *p*H 4.6.[83]

No general rule can be given for the effect of salts. Most of them accelerate the speed of mutarotation, those of alkaline reaction standing first in this respect. Sodium chloride, however, has been found by Levy[84] and also by Trey[85] to cause the mutarotation of glucose to proceed more slowly than in pure aqueous solution. Potassium chloride behaves the same way, according to Mukhin and Ass.[86]

Mutarotation of sugars takes place not only in water but also in other solvents such as absolute methyl alcohol, ethyl alcohol, acetone, etc. The change in rotation proceeds much more slowly, however, in organic solvents than in aqueous solution. This is shown in the following results by Grossmann and Bloch,[87] which give the mutarotation of several sugars in pyridine and formic acid. For the effect of pure, dry pyridine see p. 286.

Sugar	$[\alpha]_D$ in Pyridine				$[\alpha]_D$ in Formic Acid			
	After Solution		Constant		After Solution		Constant	
		min.		days		min.		days
Xylose	+117.39	8	+ 40.63	4	+ 40.34	4	+ 66.60	2
Rhamnose	− 41.39	5	− 32.77	4	+ 10.20	5	− 35.76	6
Galactose	+154.28	23	+ 59.83	3	+ 89.11	5	+127.35	5
Glucose	+149.60	10	+ 74.79	4	+ 72.16	5	+122.51	4
Fructose	−174.13	10	− 34.83	1	− 94.32	5	− 47.83	8
Maltose	+103.48	15	+123.80	11	+129.11	10	+172.15	3

A peculiarity of xylose and rhamnose in pyridine is an increase in the rotation after solution. Grossmann and Bloch observed a maximum of +122.07 in xylose 15 minutes after solution and a maximum of −45.92 in rhamnose 30 minutes after solution. It is seen that mutarotation in the two solvents proceeds in many cases in opposite directions and that there is no relation between the constant rotations and those observed in aqueous solution.

The velocity coefficients of mutarotation of several sugars dis-

[83] Isbell and Pigman, *Bur. Standards J. Research*, 20, 773 (1938).
[84] *Z. physik. Chem.*, 17, 320 (1895).
[85] *Z. physik. Chem.*, 22, 424 (1897).
[86] *J. Chim. Ukraine*, 1, 458 (1925).
[87] *Z. Ver. deut. Zucker-Ind.*, 62, 19 (1912).

solved in formamide have been measured at 20° C. by Mackenzie and Ghosh,[88] with the following results

	k
Xylose	0.00306
Glucose	0.00109
Galactose	0.00199
Mannose	0.00326
Fructose	0.00839
Maltose	0.00163

Comparison with Table XLVII, p. 285, again shows the much slower mutarotation in an organic solvent. The addition of water to solutions of sugar in organic solvents accelerates, and conversely the addition of alcohol, acetone, etc., to aqueous solutions retards, the speed of mutarotation. As a general rule the presence of any soluble nonelectrolyte, such, for example, as sucrose, will increase the time necessary for a mutarotating sugar to reach constant polarization.

Mutarotation not only takes place after dissolving reducing sugars, but also occurs upon the liberation of these sugars from higher saccharides by the action of enzymes. The phenomenon is one which the sugar chemist has always to bear in mind. Polariscopic measurements are always referred to the normal constant rotation. The latter condition may be produced almost instantly by heating the solution or by adding a little free alkali, but when such means are employed care must be taken to prevent the liability of chemical change. The safest course is to allow the solution to stand until the rotation has come to equilibrium in the natural way.

Theories of Mutarotation. Many theories have been proposed to explain mutarotation. According to the views of Landolt[89] and other authorities it was thought that the phenomenon might be due to the formation of molecular aggregates immediately after solution, which afterwards decompose into simple molecules of lower rotation. These earlier theories were largely disproved, however, by the experiments of Arrhenius,[90] and of Brown and Morris,[91] who showed that no change occurred in the molecular weight of a sugar during mutarotation. Tollens[92] and others of his school have supposed that mutarotation might be caused by the formation of unstable hydrates which, by the splitting off of water, cause a change in rotation.

[88] *Proc. Roy. Soc. Edinburgh*, 36, 204 (1916).
[89] "Das optische Drehungsvermögen," p. 58, 1879.
[90] *Z. physik. Chem.*, 2, 500 (1888).
[91] *Chem. News*, 57, 196 (1888).
[92] *Ber.*, 26, 1799 (1893).

Much additional light was thrown upon the subject in 1895 by Tanret,[93] who discovered that sugars could exist in both a high- and a low-mutarotating form. The relationship of these several modifications, according to Tanret's classification, is shown for four different sugars in the following table.

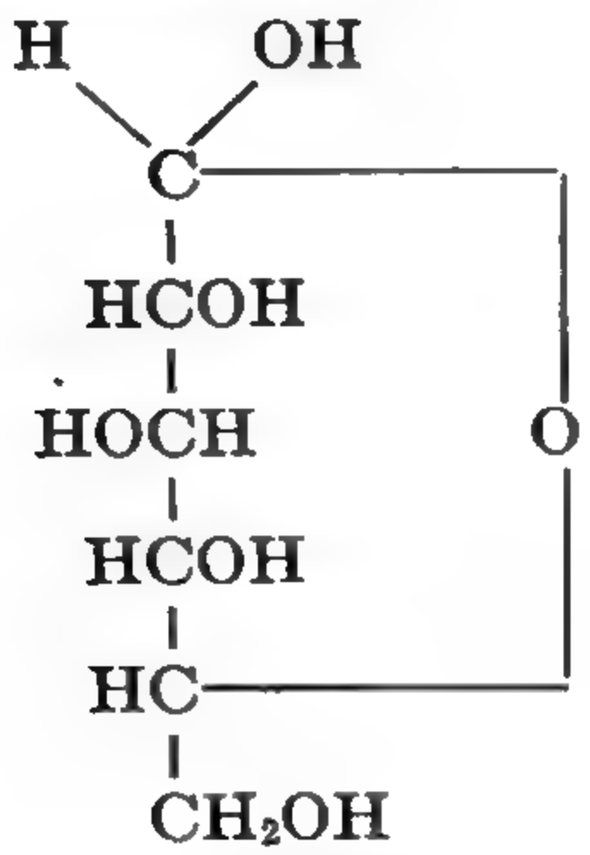
Sugar	α Metastable	β Stable	γ Metastable
d-Glucose	+105°	+52.5°	+22.5°
d-Galactose	+135	+81	+52
Lactose	+ 88	+55	+36
Rhamnose	− 6	+ 9	+23

Tanret's α modification represents the ordinary sugar as obtained by crystallization from aqueous solution. The β modification, or form of constant rotation, was usually obtained by precipitating a saturated aqueous solution of the α sugar with several volumes of absolute alcohol. The γ modification was usually prepared by evaporating a concentrated solution of the α sugar to dryness and then heating for several hours to about 100° C. Repeating the process several times increases the purity of the various modifications. In the case of rhamnose the α modification is the lower, and the γ modificatian the higher, rotating form.

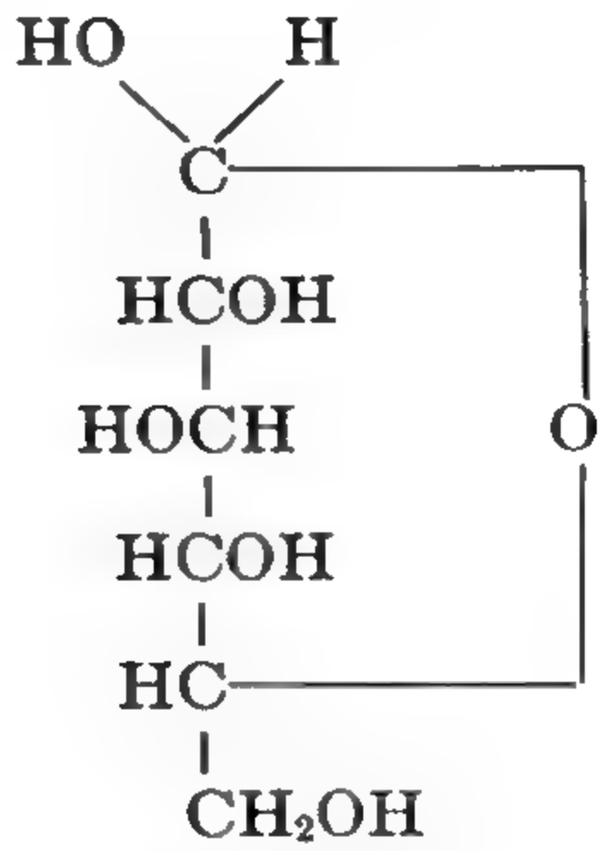
Previous to Tanret's work, Lippmann[94] had expressed the view that mutarotation might be due to a stereochemical changé between two forms of the same sugar, and showed how, by adopting a form of structure first proposed by Tollens, one of the terminal carbon atoms of the sugar molecule became asymmetric (i.e., connected to four dissimilar atoms or groups), thus permitting the existence of two configurations for the same sugar. The theory of mutarotation most generally accepted at the present time assigns one of these configurations to the high-rotating, and the other configuration to the low-rotating form. The mutarotation reaction according to Lowry[95] is thus regarded as a balanced reaction between two isomeric forms of the same

[93] *Compt. rend.*, **120**, 1060 (1895).
[94] " Chemie der Zuckerarten," 2nd ed., pp. 130, 990, 992, 1895.
[95] *J. Chem. Soc.*, **75**, 212 (1899).

sugar, as for example:



α-*d*-glucopyranose
$[\alpha]_D = +113.4$



β-*d*-glucopyranose
$[\alpha]_D = +19$

Hudson[96] proposed to designate as the α modification the most dextrorotatory form of the sugars in the *d* series, and the least dextrorotatory form in the *l* series; conversely, the β modification is the least dextrorotatory form in the *d* series and the most dextrorotatory form in the *l* series. The equilibrium mixture is termed $\alpha \rightleftarrows \beta$.

The specific rotations calculated by Hudson and Yanovsky[97] for the α and β modifications, and the equilibrium rotations of thirteen sugars, are shown in Table XLIX, water being used as the solvent.

TABLE XLIX

Sugar	α	$\alpha \rightleftarrows \beta$	β
d-Glucose	+113.4	+ 52.2	+ 19
d-Galactose	+144.0	+ 80.5	+ 52
d-Mannose	+ 34	+ 14.6	− 17
d-Fructose	− 21	− 92	−133.5
d-Xylose	+ 92	+ 19	− 20
d-Lyxose	+ 5.5	− 14	− 36
d-Arabinose	− 54	−105	−175
l-Rhamnose	− 7.7	+ 8.9	+ 54
d-Glucoheptose	+ 45	− 20.4	− 28.4
Lactose	+ 90.0	+ 55.3	+ 35
Maltose	+168	+136	+118
Melibiose	+179	+142.5	+124
Cellobiose	+ 72	+ 35	+ 16

Hudson's nomenclature, although admittedly arbitrary, has been quite generally adopted. But various attempts have been made to

[96] *J. Am. Chem. Soc.*, 31, 66 (1909).
[97] *J. Am. Chem. Soc.*, 39, 1035 (1917).

introduce a more rational system. Riiber,[98] for example, suggested that the sugars in which the hydroxyl groups on carbon atoms 1 and 2 are in the *cis* position be called α, and those where they are in the *trans* position β. Isbell has proposed a nomenclature somewhat different from Hudson's, based on the rate of oxidation of the two modifications with bromine water. He and Pigman[99] were able to show that β-*d*-glucose, in which the hydroxyl on carbon atom 1 is in the *trans* position to the oxygen ring, is much more readily oxidized to the δ-lactone of gluconic acid than α-*d*-glucose which has the hydroxyl in the *cis* position. Further investigation[100] has shown this to be true for other sugars also, and Isbell and Pigman have suggested that, when the oxygen ring lies to the right, the more dextrorotatory modification shall be designated as α and the less dextrorotatory as β, but when the oxygen ring lies to the left, the more levorotatory modification is called α and the less levorotatory one β. This system has the advantage that the designation as α or β of the pentoses and heptoses related to each of the hexoses is consistent throughout.

The actual mechanism of mutarotation has not been definitely established, but it is supposed that, in slow mutarotation, the open-chain aldehyde or keto modification is the intermediate form between the α and β forms, only a very small quantity of the carbonyl compound being sufficient to establish the equilibrium. This type of molecular rearrangement has been termed oxocyclodesmotropy by Jacobson and Stelzner.[101] According to Lippich a solution of *d*-glucose contains about 0.25 per cent of the aldehydo form, and that of other sugars as much as 1 or 2 per cent. Lippich's conclusion has been strengthened by the polarographic studies of Cantor and Peniston,[102] which indicate that the concentration of the aldehydo form is roughly proportional to the velocity constant of mutarotation of an aldose.

The proportion between the α and β modifications varies not only with the particular sugar, but also with the solvent, the total concentration, and the temperature. Equilibrated *d*-glucose solutions in water contain about 2 parts of β sugar to 1 of α sugar.

According to Isbell and Pigman,[103] the rapid type of mutarotation is entirely different in character from the slow interconversion of the α and β forms having the same ring structure. The rapid mutarota-

[98] *Tids. Kjemi Bergvesen*, **12**, 227 (1932).
[99] *Bur. Standards J. Research*, **10**, 337 (1933).
[100] *Bur. Standards J. Research*, **18**, 141, 505 (1937).
[101] Meyer-Jacobson, "Lehrbuch der organischen Chemie," 2nd ed., Vol. I, Part 2 pp. 886 ff.
[102] *J. Am. Chem. Soc.*, **62**, 2113 (1940).
[103] *J. Research Nat. Bur. Standards*, **20**, 773 (1938).

tion of fructose, galactose, etc., is essentially due to a change in the ring form from pyranose to furanose, or vice versa. When solid fructose is dissolved in water it changes from the pyranose to the furanose form until equilibrium is reached, with the same velocity as when fructofuranose is split off from sucrose by inversion and changes over to the pyranose form until equilibrium is again reached.

Hendricks and Rundle[104] have shown, however, that tetramethyl-α-*d*-galactose, which can exist only in the pyranose but not in the furanose form, also exhibits complex mutarotation. It appears therefore that other factors are involved in complex mutarotation besides the pyranose-furanose interconversion.

Since both the specific rotation and the mutarotation velocity are affected by temperature, a solution of a mutarotating sugar, quickly cooled to a lower temperature, requires time to reach the new rotation equilibrium. For this reason solutions of cane sugar, inverted at a high temperature and to be read at 20° C. in the saccharimeter, must be allowed to stand at this temperature for a sufficient time to reach rotation equilibrium.

For a review of mutarotation and its mechanism, the chemist is referred to the various special works on this subject.[105]

[104] *J. Am. Chem. Soc.*, 60, 3007 (1938).

[105] Hudson, *J. Am. Chem. Soc.*, 32, 889 (1910); Lowry, *Chem. Rev.*, 4, 231 (1928); Glasstone, "Recent Advances in Physical Chemistry," p. 331 ff.

CHAPTER IX

METHODS OF SIMPLE POLARIZATION

DETERMINATION OF SUGARS FROM ANGULAR ROTATION

The amount of a single optically active sugar, in the presence of optically inactive substances or in the presence of substances without effect upon its specific rotation, may be calculated by means of either formula for specific rotation (p. 263).

$$[\alpha]_D = \frac{100\,a}{l \times c} \quad \text{whence} \quad c = \frac{100\,a}{l \times [\alpha]_D} \tag{1}$$

$$[\alpha]_D = \frac{100\,a}{l \times p \times d} \quad \text{whence} \quad p = \frac{100\,a}{l \times d \times [\alpha]_D} \tag{2}$$

As to which of the above methods of calculation is to be used, the first or concentration formula is the better where a definite weight of substance is made up to volume before polarization, the usual method of procedure; if, however, a sugar solution of known density is polarized directly, then the second or percentage formula is to be employed.

The following formulas are given for calculating the concentration (grams per 100 ml.) of different sugars from the angular rotation (a) in a 2-dm. tube.

1 Arabinose $c = \frac{100\,a}{2 \times +104.5} = 0.4785\,a$

2 Xylose $c = \frac{100\,a}{2 \times +19.0} = 2.6316\,a$

3 Glucose $c = \frac{100\,a}{2 \times +52.8} = 0.9470\,a$

4 Fructose $c = \frac{100\,a}{2 \times -92.5} = 0.5405\,a$ (left degrees)

5 Galactose $c = \frac{100\,a}{2 \times +81.0} = 0.6173\,a$

6 Sucrose $c = \frac{100\,a}{2 \times +66.5} = 0.7519\,a$

7 Maltose $c = \frac{100\,a}{2 \times +138.0} = 0.3623\,a$

8 Lactose $c = \frac{100\,a}{2 \times +52.5} = 0.9524\,a$

9 Raffinose $(+\,5\,H_2O)$ $c = \frac{100\,a}{2 \times +104.5} = 0.4785\,a$

10 Raffinose (anhydride) $c = \frac{100\,a}{2 \times +123.15} = 0.4060\,a$

The percentage p of a sugar in solution is equal to the value of c, as expressed above, divided by the density of the solution.

Such formulas as the above are sufficiently accurate for many purposes of analysis. However, where the specific rotation of the sugar is affected by changes in concentration or temperature, the results as obtained above can be considered only approximate; to obtain the correct concentration or percentage, it is necessary to calculate the specific rotation corresponding to the approximate value of c or p at the temperature of polarization and substitute this corrected specific rotation in formula (1) or (2) for the final calculation of c or p.

Example. Fifty grams of a dextrose sirup were dissolved to 100 ml.; the constant rotation of the solution thus obtained was +34.55 circular degrees in the 200-mm. tube. Required the percentage of dextrose in the sirup.

From formula 3 we obtain by substitution $c = 0.9470 \times 34.55 = 32.72$ g. dextrose in the 100 ml. of solution or for the 50 g. of sirup, 65.44 per cent approximately. The specific rotation of dextrose for $c = 32.72$ is found from the formula $[\alpha]_D^{20^\circ} = +\,52.50 + 0.0227\,c + 0.00022\,c^2$ (p. 270) to be +53.48; substituting this in the general formula for c we obtain

$$c = \frac{100 \times 34.55}{2 \times 53.48} = 32.30 \text{ g. dextrose}$$

in the 100 ml. of solution or for the 50 g. of sirup the true percentage 64.60, which is 0.84 per cent less than the value by the uncorrected formula.

By modifying the formula for c, so as to correct for the variations in specific rotation, the labor of the second calculation in the above example may be eliminated. In the case of glucose, by calculating the angular rotation (a) for the 2-dm. tube, corresponding to concentrations ranging from 10 to 60, we obtain, using the method of least squares (p. 266), the formula $c^1 = 0.958\,a - 0.00067\,a^2$.

Example. Applying the last formula to the previous example, we obtain, for c, 32.299 g. dextrose in the 100 ml. of solution, or for the 50 g. sirup 64.60 per cent.

[1] For p Landolt gives the formula $p = 0{,}948\,a - 0.0032\,a^2$. ("Das optische Drehungsvermögen," 2nd ed., p. 447, 1898.)

DETERMINATION OF SUGARS FROM SACCHARIMETER READINGS

Conversion of Saccharimeter Readings into Angular Rotation. The general methods of optical analysis just described are more especially applicable to polarimeters, where readings are taken in angular degrees; the formulas given are equally applicable, however, to saccharimeters, the scale reading of which must be converted into angular degrees by means of the proper conversion factor. For general purposes the factor established for sucrose may be applied to other sugars. In the case of the Ventzke scale, sugar degrees $\times$ 0.34657 = angular rotation, for the D line, according to Herzfeld and Schönrock, or 0.3462 according to Bates and Jackson for the corrected 26.000-g. scale. Since, however, the rotation dispersion of the various sugars, with reference to the quartz compensation of the saccharimeter, may differ somewhat from that of sucrose, it is always better, where exact data are available (which is unfortunately not always the case), to use the conversion factor established for the particular sugar. For a few sugars Landolt[2] has established the following factors for converting divisions of the Ventzke scale into circular degrees.

Sucrose	0.3465
Lactose	0.3452
Glucose	0.3448
Invert sugar	0.3432
Raffinose	0.3450

Brown, Morris, and Millar[3] give the following:

Sucrose, 10 per cent solution	0.3469
Maltose, 10 per cent solution	0.3449
Maltose, 5 per cent solution	0.3457
Glucose, 10 per cent solution	0.3442
Glucose, 5 per cent solution	0.3454
Starch products, 10 per cent solution	0.3458
Starch products, 5 per cent solution	0.3454

Zerban[4] compared the angular rotation in unpurified sodium light with the reading on a saccharimeter calibrated according to the Herzfeld-Schönrock scale, using bichromate-filtered light, and found these conversion factors:

Invert sugar, about 13 g. in 100 ml.	0.3462
Invert sugar, about 69 g. in 100 ml.	0.3460
Fructose, about 10 g. in 100 ml.	0.3474

[2] *Ber.*, 21, 194 (1888).
[3] *J. Chem. Soc. Trans.*, 71, 92 (1897).
[4] *J. Am. Chem. Soc.*, 47, 1104 (1925); fructose factor unpublished.

Conversion factors for the Bates-Jackson scale are obtained by multiplying those for the Ventzke scale by 0.999.

Herzfeld,[5] with a solution containing 11.29 per cent anhydrous maltose, obtained upon a Peters saccharimeter, using a Welsbach light with chromate filter, a reading of 93.88 Ventzke degrees at 20° C., and with the same solution upon a Lippich polarimeter a reading of 32.60 circular degrees at 20° C. The value of a Ventzke-scale division for maltose under these conditions is therefore $32.60 \div 93.88 = 0.3471$ circular degree, a figure perceptibly greater than the values of Brown, Morris, and Millar. Differences in concentration of the sugar solutions examined but more especially differences in the polarizer and in the optical center of gravity of the light employed for illuminating the saccharimeter are the chief causes of such discrepancies. The chemist, therefore, should employ any prescribed conversion factor with caution and use it only under the conditions for which it was established. It is also well to verify a conversion factor wherever possible by comparative readings of the same sugar solution upon a polarimeter. The polarimeter does away with the errors of rotation dispersion and, aside from the objection of using monochromatic light, is always to be preferred in methods where the concentration or percentage of sugar is calculated from the angular rotation. If a quartz-wedge saccharimeter is the only instrument available, the average factor 0.346 may be used for most purposes without serious error.

Normal Weights of Sugars. If a normal weight of each particular sugar is taken for polarization (i.e., the weight of pure sugar which dissolved to 100 ml. will give a scale reading of 100), the percentage (uncorrected) of sugar may be read directly upon the saccharimeter.

There are a number of methods of calculating the normal weight for different sugars. If we assume in case of the Ventzke scale that the angular rotation of each division is 0.34657 circular degree for all sugars, then the normal weight (20° C., 100 ml.) of any sugar, for the 2-dm. observation tube, as compared with 26.026 g., will be inversely proportional to the specific rotations of this sugar and of sucrose, that is:

$$[\alpha]_D^{20} : 66.54 :: 26.026 \text{ g.} : X; \text{ whence } X \text{ (the normal weight)} = \frac{1732}{[\alpha]_D^{20}}$$

The normal weights of several sugars calculated by this method are given in the following table:

[5] *Ber.*, **28**, 441 (1895).

TABLE L

NORMAL WEIGHTS OF DIFFERENT SUGARS FOR VENTZKE SCALE

Sugar	Specific Rotation $[\alpha]_D^{20}$	Normal Weight
Glucose	+ 53.46 c = 32.5 g.	$\frac{1732}{53.46}$ = 32.398 g.
Fructose	− 94.05 c = 18.42 g.	$\frac{1732}{94.05}$ = 18.416 g.
Invert sugar	− 20.07 c = 10.0 g.	$\frac{1732}{20.07}$ = 86.333 g.
Lactose (+H_2O)	+ 52.53	$\frac{1732}{52.53}$ = 32.972 g.
Maltose	+138.25 c = 12.5 g.	$\frac{1732}{138.25}$ = 12.528 g.
Raffinose (+5 H_2O)	+104.5	$\frac{1732}{104.5}$ = 16.574 g.
Raffinose (anhydride)	+123.17	$\frac{1732}{123.17}$ = 14.062 g.

If the Bates-Jackson scale, with a normal weight of 26.000 g. sucrose, is used as the basis, all the normal weights given above must be multiplied by 0.999.

Although the normal weights calculated in this manner are sufficiently exact for most purposes of analysis they must not be regarded as absolute. Owing to the differences previously mentioned in rotation dispersion for the different sugars the angular rotation of each Ventzke-scale division will vary slightly from 0.34657 circular degree with a corresponding change in the value of the normal weight.

If the value of the 100° saccharimetric reading of each sugar has been established in circular degrees, for the same conditions under which analyses are made, it is always better to base the calculation of the normal weight upon this. The method of calculation for the Ventzke scale, using as illustrations four of the sugars previously taken, is as follows:

From the general formula $c = \frac{100\,a}{l \times [\alpha]_D}$ we obtain for

Glucose
(1° V. = 0.3448 circular degree, Landolt), $c = \frac{100 \times 34.48}{2 \times 53.46} = 32.248$ g.

Fructose
(1° V. = 0.3474 circular degree, Zerban), $c = \frac{100 \times 34.74}{2 \times 94.05} = 18.469$ g.

Invert sugar
(1° V. = 0.3460 circular degree, Zerban), $c = \frac{100 \times 34.60}{2 \times 20.07} = 86.197$ g.

Lactose

(1° V. = 0.3452 circular degree, Landolt), $c = \frac{100 \times 34.52}{2 \times 52.53} = 32.857$ g.

Maltose

(1° V. = 0.3449 circular degrees, {Brown, Morris, and Millar}) $c = \frac{100 \times 34.49}{2 \times 138.25} = 12.474$ g.

Raffinose +5 H_2O

(1° V. = 0.3450 circular degree, Landolt), $c = \frac{100 \times 34.50}{2 \times 104.5} = 16.507$ g.

The conversion factors to be employed, and hence the values of the normal weights, will necessarily depend upon the quality of the light used for illuminating the saccharimeter and upon the type of polarizer. The value of a saccharimeter division in circular degrees for a solution of the sugar of the approximate concentration, therefore, should be established by the chemist himself wherever possible for his own particular instrument. Each type of quartz-wedge saccharimeter has its own individual optical peculiarities which produce variable effects with different observers. It is partly for this reason that so many discrepancies have arisen in the values of normal weights and in the circular-degree equivalents of saccharimeter scales. The recalculation of the normal weights of French saccharimeters of the Laurent type from values obtained with instruments having a Lippich polarizer is thus open to criticism. The only valid procedure is for each observer to determine the weight of carefully purified sugar that will give a reading of exactly 100 on the scale of the saccharimeter being employed and then to determine the circular-degree equivalent of this by reading the same tube of sugar solution in an accurate polarimeter under the prescribed conditions of temperature and wavelength of light. The average of the results thus obtained by the greatest number of skilled observers will give the nearest approach to the most accurate value.

Jackson[6] has, by direct measurement, determined the normal weight of glucose for the Bates-Jackson scale to be 32.231 g., which corresponds to 32.264 g. for the Herzfeld-Schönrock scale. For the normal weight of fructose Jackson and Mathews[7] found 18.407 g. on the Bates-Jackson scale, or 18.425 g. on the Herzfeld-Schönrock scale.

Correction for Concentration and Temperature. When normal weights of the different sugars are used, the observed saccharimeter readings require correction for changes in concentration and temperature as described on p. 295. Where much work is done with a single sugar a table of corrections should be prepared, giving the actual sugar value corresponding to each scale division of the saccharimeter. The

[6] *Bur. Standards Sci. Paper* 293, p. 633, 1916.

[7] *Bur. Standards J. Research,* 8, 403 (1932).

correction table for sucrose (p. 185) or the following results calculated by Browne[8] for glucose upon the basis of the normal weight of 32.25 g. will illustrate the method. The corrections found by Jackson, from saccharimetric data, are given in the last column of the table, for the normal weight of 32.264 g. (Herzfeld-Schönrock scale).

Scale Division	Concentration, Grams Glucose 100 ml. 20° C.	Specific Rotation, Glucose $[\alpha]_D^{20}$	Actual Glucose Value of Scale Division	Correction to Be Added	
				Browne	Jackson
100	32.250	53.46	100.00	0.00	0.00
90	29.025	53.34	90.20	0.20	0.20
80	25.800	53.23	80.35	0.35	0.35
70	22.575	53.12	70.45	0.45	0.46
60	19.350	53.02	60.50	0.50	0.53
50	16.125	52.92	50.51	0.51	0.55
40	12.900	52.83	40.48	0.48	0.53
30	9.675	52.74	30.41	0.41	0.46
20	6.450	52.66	20.30	0.30	0.35
10	3.225	52.58	10.17	0.17	0.20
1	0.323	52.51	1.02	0.02	....

The correction necessary to be added to any reading (s) of the saccharimeter scale, as formulated from the above table, is equal very closely to $+0.02\,s - 0.0002\,s^2$. The percentage of glucose (G) corresponding to any scale reading (s) of the saccharimeter, therefore, is expressed by the formula

$$G = s + 0.02\,s - 0.0002\,s^2 \text{ (Herzfeld-Schönrock scale)}$$

A table similar to the one given above for glucose has been calculated by Jackson and Mathews for fructose, at 20° and at 25° C. The normal weight taken is 18.407 g. at 20° C., and 19.003 g. at 25° C. (Bates-Jackson scale). The corrections are to be added to the negative readings found, increasing them to higher negative values (see p. 301).

Some authorities have established the normal weights of sugars for 5, 10, 15, 20, and 25 per cent solutions. Landolt[9] gives as the normal weight of glucose for a 5 per cent solution 32.91 g., for a 15 per cent solution 32.75 g., and for a 25 per cent solution 32.50 g., in which connection he states that, in weighing out the glucose-containing material for polarization, the chemist must select his normal weight accord-

[8] *J. Ind. Eng. Chem.*, 2, 526 (1910).

[9] Landolt, " Das optische Drehungsvermögen," 2nd ed., p. 448, 1898.

Scale Division	Correction		Scale Division	Correction	
	20° C.	25° C.		20° C.	25° C.
100	0.00	0.00	50	0.57	0.61
95	0.11	0.12	45	0.56	0.60
90	0.21	0.23	40	0.54	0.57
85	0.30	0.33	35	0.51	0.55
80	0.38	0.40	30	0.47	0.50
75	0.44	0.47	25	0.42	0.45
70	0.50	0.52	20	0.35	0.39
65	0.53	0.56	15	0.28	0.30
60	0.56	0.59	10	0.19	0.21
55	0.57	0.61	5	0.10	0.11
52	0.58	0.61	0	0.00	0.00

ing to the amount of glucose present. This, of course, involves a preliminary assay of the material under examination, which means practically doubling the work of analysis. A variable normal weight, moreover, is confusing and a source of error. Wherever possible one fixed value should be given to the normal weight, the value to be selected (as for sucrose) being that weight of chemically pure sugar which, dissolved to 100 ml. and polarized at 20° C. in a 200-mm. tube, will give a constant reading of exactly 100 upon the saccharimeter. If in the use of such a normal weight with impure products, readings of less than 100 are obtained, they are corrected by a table or formula similar to those given above.

Conversion of Saccharimeter Readings into Weight of Sugars. It is often desirable to express the equivalent of a saccharimeter reading, for a 200-mm. tube, in grams of a particular sugar in 100 ml. This equivalent can be found by multiplying the values of the formulas on p. 294 by the angular rotation of 1° of the saccharimeter scale, thus:

1° angular rotation D		= 0.4785 g. arabinose
1° Ventzke sugar scale	= 0.4785 × 0.34657	= 0.1658 g. arabinose
1° French sugar scale	= 0.4785 × 0.21667	= 0.1037 g. arabinose

Owing to the lack of absolute agreement in the value of each saccharimeter scale in circular degrees, due to rotation dispersion, variation in quality of light, etc., the equivalent of 1° of a saccharimeter scale is best expressed as $\frac{1}{100}$ of the weight of sugar, which will give a reading of 100° under the prescribed conditions of analysis (i.e., $\frac{1}{100}$ of its normal weight). The correction for concentration is afterwards applied as indicated above.

The approximate value of 1° V. for the more common sugars is

given below.

WEIGHT OF SUGAR IN 100 ML.

1° V. at 20° C. = 0.2603 g. sucrose
1° V. at 20° C. = 0.3226 g. glucose
1° V. at 20° C. = 0.1843 g. fructose
1° V. at 20° C. = 0.3297 g. lactose hydrate
1° V. at 20° C. = 0.1253 g. maltose
1° V. at 20° C. = 0.1657 g. arabinose
1° V. at 20° C. = 0.9109 g. xylose
1° V. at 20° C. = 0.2137 g. galactose
1° V. at 20° C. = 0.8633 g. invert sugar
1° V. at 20° C. = 0.1657 g. raffinose hydrate

Use of One Normal Weight for All Sugars. For many laboratory purposes it is convenient to employ but one fixed normal weight for all saccharimetric work. In such cases the normal weight of sucrose is usually taken, the percentage of each particular sugar being calculated from the scale reading by means of an appropriate factor.

The constant polarizations in degrees Ventzke of a normal weight of 26.026 g. of different sugars, when dissolved to 100 ml. and polarized in a 200-m. tube, are given in Table LI. The values are calculated only to the nearest 0.5°, which is sufficiently exact when the variations due to change in concentration are considered.

If no other optically active substances are present, the scale reading (V.°) of 26.026 g. of the sugar-containing substance multiplied by 100 and divided by the corresponding polarizing power of the pure sugar will give the percentage of sugar present. Owing to the changes in specific rotation with varying concentration, the percentages thus calculated will not be absolutely exact.

TABLE LI

VENTZKE READING OF 26.026 G. OF DIFFERENT SUGARS IN 100 ML.

Sugar	$[\alpha]_D^{20°}$ 26.026 g. in 100 ml.	Calculated Reading V° $\frac{[\alpha]_D^{20°}}{66.5} \times 100$
Sucrose	+ 66.5	+100
Arabinose	+104.5	+157
Xylose	+ 19.6	+ 29.5
Glucose	+ 53.1	+ 80
Fructose	− 94.7	−142
Invert sugar	− 20.8	− 31
Galactose	+ 81.8	+123
Maltose	+138.0	+207.5
Lactose (H_2O)	+ 52.5	+ 79
Raffinose ($5H_2O$)	+104.5	+157
Raffinose (anhydride)	+123.2	+185

TECHNICAL METHODS OF SACCHARIMETRY

The saccharimeter is most generally employed in the analysis of products of the cane- and beet-sugar industry. It must be borne in mind, however, that the readings of the saccharimeter scale indicate percentages of sucrose only if other constituents have no effect upon the scale reading; the results obtained with impure products are, therefore, more correctly expressed as degrees polarization or degrees sugar scale. For a more accurate determination of sucrose by the saccharimeter, the method of inversion must be used which will be described in the following chapter.

Methods for Polarizing Raw Sugars

Rules of the International Commission. The rules of the International Commission for Unifying Methods of Sugar Analysis[10] are as follows:

In general all polarizations are to be made at 20° C.

The verification of the saccharimeter must also be made at 20° C. For instruments using the Ventzke scale 26 g. of pure dry sucrose, weighed in air with brass weights, dissolved to 100 metric cc. at 20° C. and polarized in a room, the temperature of which is also 20° C., must give a saccharimeter reading of exactly 100.00. The temperature of the sugar solution during polarization must be kept constant at 20° C.

For countries where the mean temperature is higher than 20° C., saccharimeters may be adjusted at 30° C. or any other suitable temperature, under the conditions specified above, provided that the sugar solution is made up to volume and polarized at this same temperature.

In effecting the polarization of substances containing sugar employ only half-shade instruments.

During the observation keep the apparatus in a fixed position and so far removed from the source of light that the polarizing Nicol is not warmed.

As sources of light employ lamps which give a strong illumination such as triple gas burner with metallic cylinder, lens and reflector; gas lamps with Auer (Welsbach) burner; electric lamp; petroleum duplex lamp; sodium light.

Before and after each set of observations the chemist must satisfy himself of the correct adjustment of his saccharimeter by means of standardized quartz plates. He must also previously satisfy himself of the accuracy of his weights, polarization flasks, observation tubes and cover glasses. (Scratched cover glasses must not be used.) Make several readings and take the mean thereof, but no one reading may be neglected.

[10] *Proceedings of Paris Meeting*, July 24, 1900.

The rules for preparing the solutions for polarization were amended at the Eighth Session of the International Commission, in 1932, and read as follows:[11]

Single Polarization. To make a polarization, the whole normal weight for 100 ml. shall be used, or a multiple or fraction thereof for any corresponding volume.

As clarifying and decolorizing reagents there may be used: lead subacetate solution or Horne's dry subacetate of lead, and salt-free alumina cream. Boneblack and decolorizing powders are excluded.

After bringing the solution exactly to the mark and after wiping out the neck of the flask with filter paper, all of the well-shaken[12] clarified sugar solution is poured upon an air-dry rapidly filtering filter. During the filtration the funnel shall be covered with a watch glass or plate to prevent evaporation. At least the first 25 ml. of the filtrate is to be thrown away, and the remainder, which must be perfectly clear, is to be used for polarization.

It is understood that lead subacetate solution and alumina cream are added before making up to the mark, but Horne's dry subacetate of lead after completing the volume.

The following further provision was adopted at the Ninth Session of the Commission in 1936:[13]

If no change in the sugar scale is or has been made, clarification shall be effected with standard lead subacetate solution (Third Session of International Commission, Paris, 1900); but if a change from the Herzfeld-Schönrock scale to the International Sugar (Bates-Jackson) scale is made, then clarification shall be effected with standard dry lead subacetate (Horne's dry lead).

Methods of the New York Sugar Trade Laboratory. Details of manipulation for the above rules are left largely to individual preference or requirement. The course of operations pursued by the New York Sugar Trade Laboratory, where rapidity as well as accuracy is required, is as follows:

Weighing. Twenty-six grams of raw sugar is weighed out in a nickel sugar dish provided with a counterpoise (Figs. 160 and 169). The sugar is stirred with a horn spoon, and approximately the normal weight is transferred to the dish. The final adjustment is then made with the dish upon the scale pan of the balance, a little sugar being added or

[11] *Intern. Sugar J.*, 35, 62 (1933).

[12] Dymond (*Intern. Sugar J.*, 33, 295) has shown experimentally that insufficient mixing leads to serious errors.

[13] *Intern. Sugar J.*, 39, 32s (1937).

removed until the exact weight is secured. The danger of spilling sugar upon the scale pan during the weighing is thus largely avoided. The weighing is performed as rapidly as possible to avoid loss from evaporation of moisture and does not usually consume more than a minute of time.

Transferring. The 26 g. of sugar in the nickel dish is poured into a large funnel placed in a sugar flask; any sugar adhering to the dish and funnel is then washed into the flask with distilled water, the funnel being thoroughly rinsed inside and outside around the bottom to insure the complete removal of all sugar to the flask. From 50 to 60 ml. of water is sufficient to effect the transference.

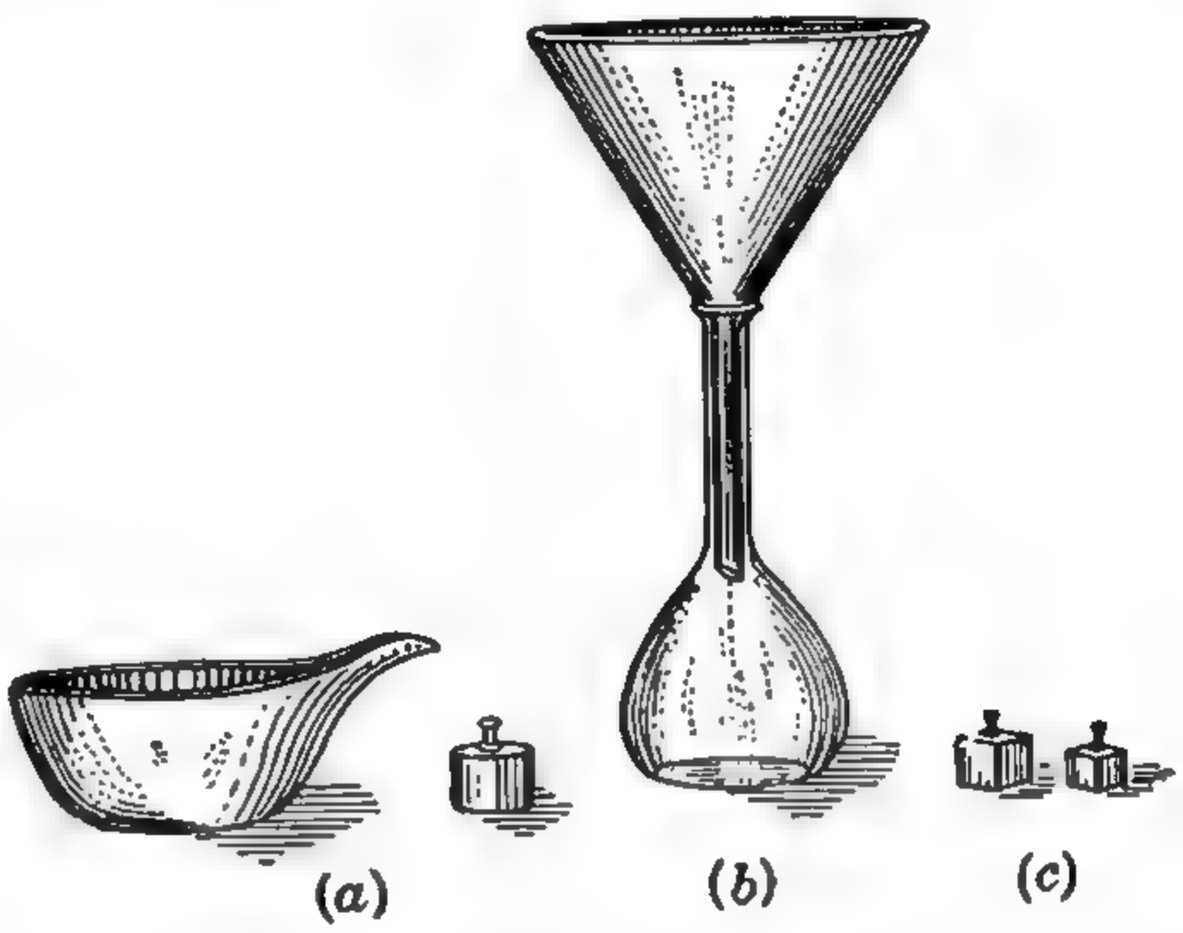


FIG. 169. (*a*) Nickel weighing dish and counterpoise. (*b*) Funnel for transferring sugar. (*c*) Normal (26.000 g.) and half-normal (13.000 g.) sugar weights.

The funnels employed in transferring the sugar are of German silver, and have a mouth 4 in. (10 cm.) in width and 3 in. (7.5 cm.) in depth, and a stem 3 in. (7.5 cm.) in length. The inner diameter of the stem (8½ mm.) is sufficiently large to allow a free passage of the sugar into the flask and the outer diameter (10 mm.) sufficiently small to allow the escape of air from the flask (see Fig. 169).

Dissolving. The solution of the sugar in the flasks is performed by means of a mechanical shaker. The machine employed in the New York Sugar Trade Laboratory is a modification of the Camp shaker used in iron and steel laboratories (Fig. 170).

The metal disk of this shaker is replaced by a circular piece of oak ⅞ in. thick, of the same diameter and of about the same weight, and containing 12 holes 2⅛ in. in diameter, each large enough to accommodate the bottom of a sugar flask. Six extra gripping devices are inserted in the collar of the shaker, thus giving 12 grips in all to hold the necks of the flasks. The collar is adjusted so as to bring the grips at the right height and exactly over the centers of the circular holes in the wooden disk. The bottoms of the flasks are inserted in the holes, and, by pressing the necks against the springs of the grips, the flasks are snapped quickly and securely into position. The shaker is connected with a small ⅓-horsepower electric motor, provided with a rheostat,

and the speed of its driving wheel is gradually brought up to 120 to 130 revolutions per minute. At this speed, solution of sugar in the flasks, using 50 to 60 ml. of water, is effected in 5 to 10 minutes, according

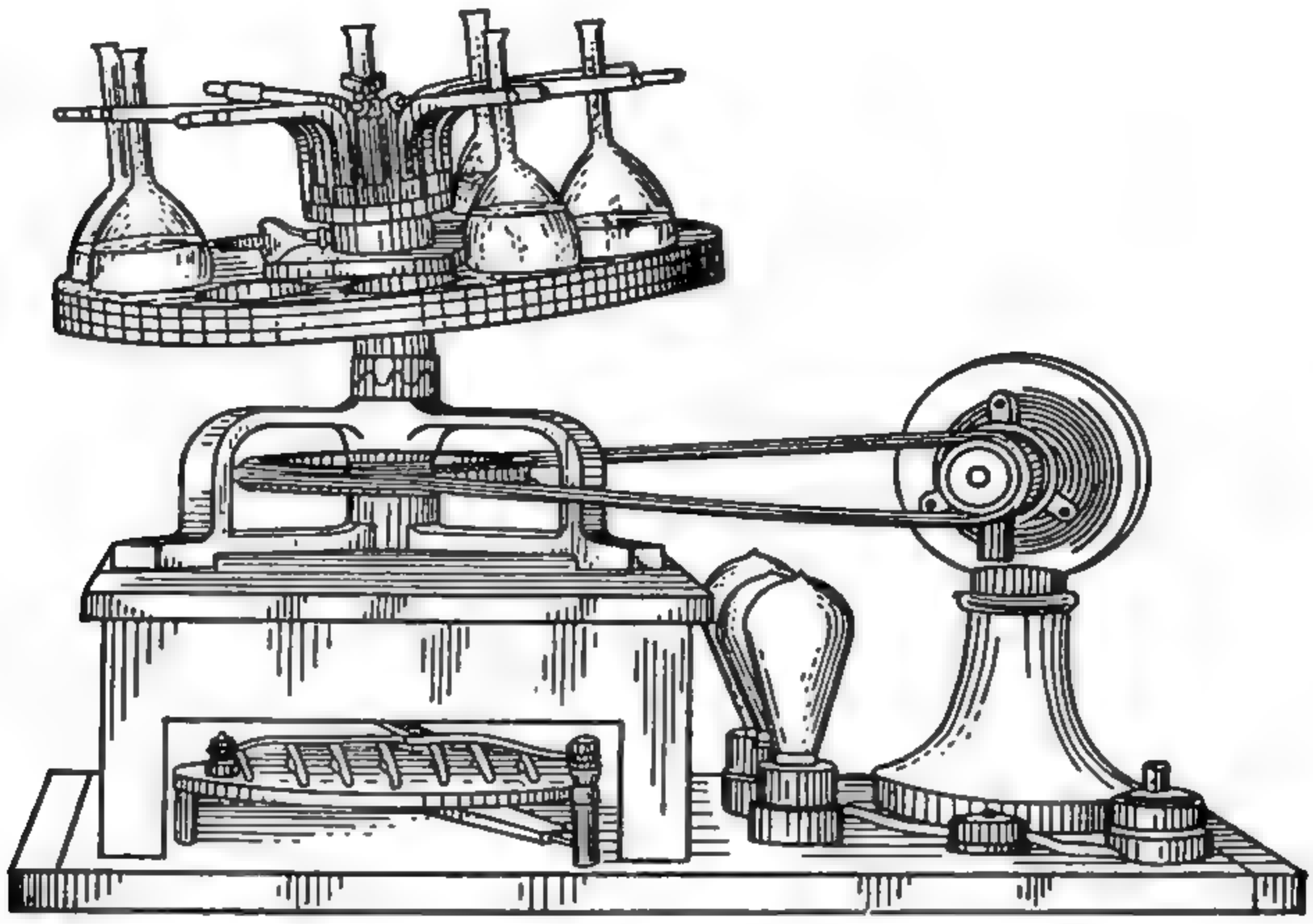


FIG. 170. Mechanical shaker for dissolving sugars.

to the size of grain, stickiness of sample, etc. If too much water is used in transferring the sugar, less motion is given to the body of the liquid, and a longer time is required to effect solution.

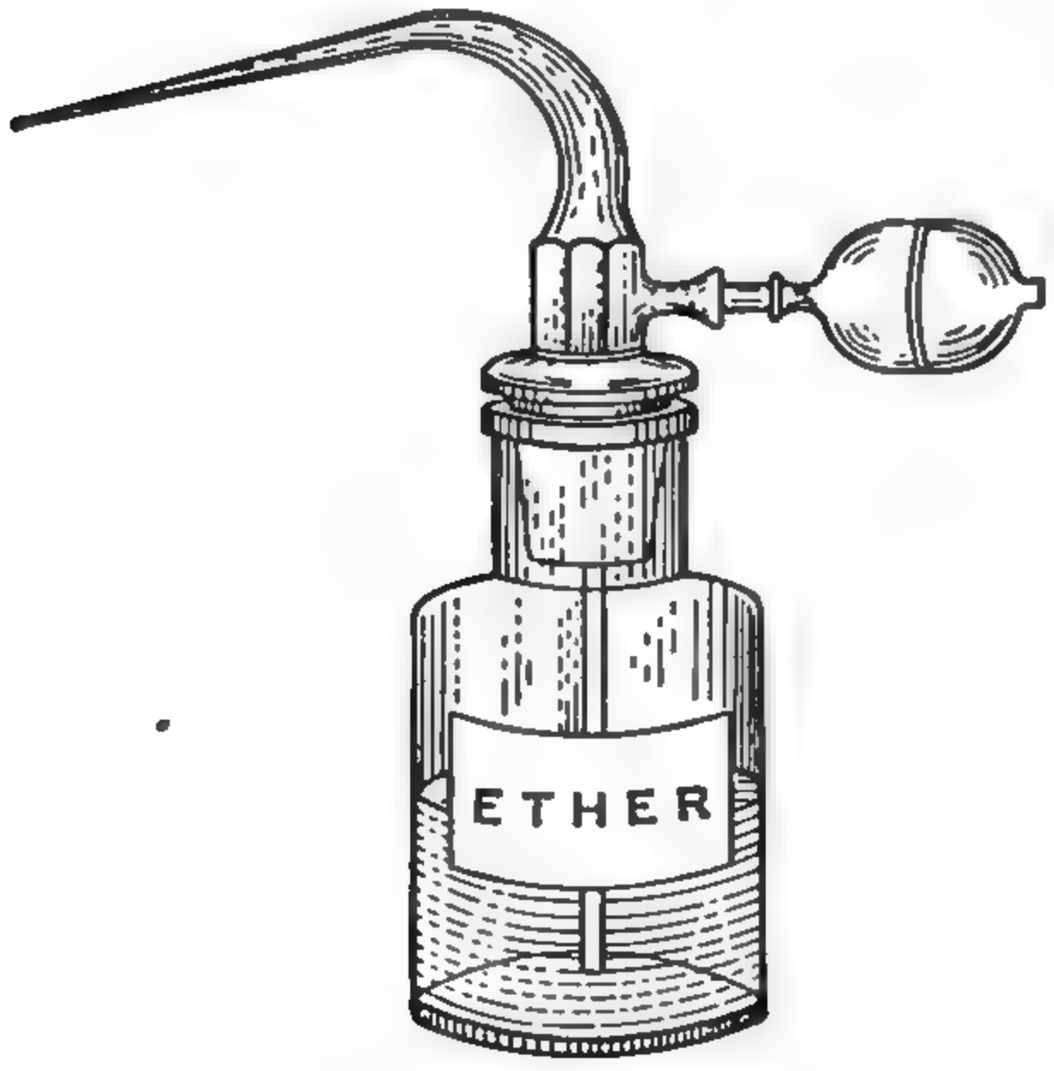


FIG. 171. Ether atomizer.

Clarifying. The solution is then clarified with the requisite amount of lead subacetate solution (sp. gr. 1.25), but no more than the amount necessary to secure a clear polariscope reading is ever employed. As a rule not over 1 ml. of the lead subacetate solution is used for high-grade centrifugal sugars, not over 1 to 2 ml. for dark centrifugal sugars, and from 2 to 6 ml. for molasses sugars. Excess of lead solution increases the polarization very markedly, and strict observance is paid to the rule of minimum quantity necessary for clarification. After the lead solution 2 ml. of alumina cream is added, the contents of the flask are well mixed, and the volume of liquid is made up to 100 ml., after sufficient time is allowed for any air bubbles to arise which may have

been occluded in the lead precipitate. Foam and air bubbles adhering to the surface of the liquid in the neck of the flask are broken up with a fine spray of ether before the volume is adjusted to the graduation mark. A small bulb atomizer (Fig. 171) is convenient for removing foam.

The distilled water used in all the work is supplied through rubber tubing from a large bottle placed at an elevation above the laboratory table. The outlets of the rubber tubes are fitted with pinchcocks and glass tips of large and fine opening, the former being used for transferring the sugar and the latter for setting the meniscus. The adjustment of the meniscus to the graduation mark is the same as that used in calibration (Fig. 165). The distilled water used for solution is kept as nearly as possible at 20° C., and the completion of the volume of sugar solution to 100 ml. is always made with the contents of the flask at this temperature.

Filtering. The contents of the flasks after thorough mixing are poured upon plaited filters in stemless funnels resting in ½-pint jars or cylinders (Fig. 166). All glassware is thoroughly cleaned and dried before using. Plaited filters, large enough to hold the entire contents of the flask, are employed.[14] The funnels are covered with watch glasses during filtration to prevent evaporation.[15] The first runnings (at least 25 ml.) of the filtrate are rejected, and the remainder is polarized at 20° C.

Mutarotation in the Polarization of Raw Sugars. Mutarotation is often observed in the polarization of honeys or highly concentrated invert sugar sirups from which glucose has crystallized or in which it exists in the state of supersaturation. Wiley and Browne have found that raw cane sugar may also exhibit mutarotation. When such sugars undergo extensive deterioration, especially of the type produced by torulae, and then dry out, the polarization directly after dissolving may be as much as 0.7° V. higher than after rotation equilibrium has

[14] For many years the filter papers used at the New York Sugar Trade Laboratory were dried in a hot-water oven and kept in a desiccator until used, because the International Commission prescribed the use of dry filter paper. Hardin (*Ind. Eng. Chem.*, 16, 1175), as well as Vnuk (*Z. Zuckerind. čechoslovak. Rep.*, 51, 125, 133), has shown, however, that dry filter paper causes an increase in polarization, and paper saturated with moisture a decrease, unless at least 25 ml. of the filtrate is rejected. For this reason the Rules of the International Commission were changed in 1932, and they now specify the use of air-dry filter paper, and rejection of at least 25 ml. of filtrate.

[15] Bates and Phelps (*Bur. Standards Scientific Paper* 221) found that a raw sugar solution for a 10-minute filtration through an uncovered funnel gave an increase of 0.02 in polarization; with half of the solution poured back the increase was 0.05, and with all the solution poured back the increase was 0.07. No increase in polarization was noted when the funnel was covered.

been reached. Two typical examples, investigated at the New York Sugar Trade Laboratory, are given here:

	Sugar A	Sugar B
Polarization 1 minute after solution....	90.85	90.15
Polarization 15 minutes after solution..	90.60	89.85
Polarization 30 minutes after solution..	90.45	89.70
Polarization 90 minutes after solution..	90.30	89.55
Polarization 3 hours after solution.....	90.15	89.50
Polarization 20 hours after solution....	90.15	89.50
Total loss in polarization.....	0.70	0.65

If the tubes are allowed to stand for 3 hours before polarizing deteriorated sugars, no discrepancies in test need be feared from mutarotation.

Polarization of Juices, Sirups, Molasses, Massecuites, etc. The method of polarization just described for sugars may be applied with minor modifications to the juices of the sugar cane, sugar beet, sorghum, and other plants, and also to sirups, molasses, massecuites, and other water-soluble products.

Sucrose Pipette. In analyzing sugar-containing juices the work may be lightened considerably by the use of Spencer's or Crampton's sucrose pipette shown in Fig. 172. This pipette is graduated upon the stem with divisions, divided into tenths, reading from 5 to 25. The pipette is so calibrated that the volume of juice delivered from the division into the stem, which corresponds to its degree Brix, is exactly a double normal weight. The pipette is constructed either for Mohr cubic-centimeter or true cubic-centimeter flasks, delivering 52.096 g. and 52.000 g. of juice, respectively. The method of employing the pipette is thus described by Meade.[16]

FIG. 172. Spencer's sucrose pipette.

Determine the density of the juice with a Brix hydrometer, noting the degree Brix without temperature correction. Fill the pipette with juice to the mark corresponding with its observed degree Brix, and discharge it into a 100-ml. flask. Add 3 to 5 ml. of diluted lead-subacetate solution, complete the volume to 100 ml. with water, mix thoroughly and filter the contents of the flask. Polarize the filtrate, using a 200-mm. tube, and divide the polariscope reading by 2 to obtain the percentage of sucrose. The juice should not be expelled

[16] "Spencer's Handbook for Cane Sugar Manufacturers," 7th ed., by Meade, p. 313, 1929.

from the pipette by blowing, and sufficient time should be allowed for thorough drainage.

The sucrose pipette may be used in connection with juices preserved with Horne's dry lead or with juices clarified by the lead salt by making the measurement after filtration at the temperature of the Brix observation. It is to be understood that the Brix is to be taken in a portion of the juice preserved with formaldehyde or mercuric chloride.

The calibration of pipettes should be verified against a balance. A volume of sugar solution corresponding to an uncorrected degree Brix should be measured in the pipette. If the instrument is correctly graduated it should deliver two normal weights of the solution (52.096 g. or 52 g. depending on which flask it is intended to be used with).

It is not advisable to use these pipettes with liquids of a higher density than 25° Brix or of greater viscosity than cane juice. These pipettes are usually used in the analysis of miscellaneous samples of juice and in the rapid testing of diluted massecuites and molasses for guidance in the vacuum-pan work. They should be frequently cleaned with a strong solution of chromic acid in sulfuric acid.

Method of Schmitz. Another procedure that dispenses with the use of normal weights, and is more generally used than the Spencer pipette, is the method of Schmitz.[17] In the original process 100 Mohr cc. of juice were measured into a 100–110-cc. flask, the required amount of lead subacetate solution was added, the volume completed to 110 cc., the solution well mixed, filtered, and polarized. The polarization P was calculated from the reading R at 17.5° C. by the formula

$$P = \frac{1.1\,R \times 26.048}{\text{sp. gr.}\,{}^{17.5°}_{17.5°} \times 100}$$

The Brix was determined in another portion of the juice with a spindle calibrated at 17.5° C., and the corresponding specific gravity was substituted in the formula. In order to avoid calculations, Schmitz computed a table based on the formula and showing directly the polarization of the juice for the reading found and for the uncorrected Brix obtained at the temperature of the polariscope reading. In this table of Schmitz the polarization was corrected for the change with change of concentration.

With the introduction of the normal temperature of 20° C. and the normal weight of 26.000 g. in 100 ml. the above formula had to be changed to

$$P = \frac{1.1\,R \times 26}{99.718 \times \text{sp. gr.}\,{}^{20°}_{20°}}$$

[17] *Z. Ver. deut. Zucker-Ind.*, **30**, 899 (1880).

where 99.718 is the weight in grams of 100 ml. of water at 20° C. If the new corrected normal weight of 26.026 g. is employed, this must be substituted for 26 in the formula. Paar[18] has published a table for this new normal weight and for uncorrected Brix readings obtained with a spindle calibrated at 20° C. In this table the change of polarization with concentration has not been considered because it is too small to cause an appreciable error. Other Schmitz tables are to be found in textbooks on factory control.

For the analysis of highly concentrated sugar products, such as sirups, molasses, and massecuites, the normal weight of substance is weighed out as with raw sugar. With very dark-colored molasses and massecuites, it is often necessary to make the normal weight of substance after clarification up to 200 ml. instead of 100 ml. in order to reduce the depth of color sufficiently to polarize in a 200-mm. or even, at times, in a 100-mm. tube. The reading thus obtained is multiplied by 2 (or if polarization is made in a 100-mm. tube by 4) to obtain the true direct polarization.

CLARIFYING AGENTS AND ERRORS ATTENDING THEIR USE

In the clarification of dark-colored molasses and other sugar-house products a much larger amount of clarifying agent must be used than is necessary with raw sugars, juices, and other substances of high purity. The employment of excessive quantities of clarifying agent, however, introduces serious errors in the work of polarization. For convenience these errors will be considered under the following heads:

I. Errors due to the volume of precipitated impurities.
II. Errors due to precipitation of sugars from solution.
III. Errors due to change in specific rotation of sugars and non-sugars.

The influence of these errors will first be considered in connection with the different acetates of lead, which are the salts most generally used for clarification.

Acetates of Lead. Three well-characterized acetates of lead[19] have been isolated in the crystalline form. These are (1) the normal or neutral acetate of lead $Pb(C_2H_3O_2)_2 \cdot 3\,H_2O$; (2) the basic acetate $3\,Pb(C_2H_3O_2)_2 \cdot PbO \cdot 3\,H_2O$; (3) the basic acetate $Pb(C_2H_3O_2)_2 \cdot 2\,PbO \cdot 4\,H_2O$. The clarifying power of solutions of these acetates is in general proportionate to the content of basic PbO. The normal acetate, al-

[18] *Deut. Zuckerind.*, 57, 897, 908 (1932).
[19] R. F. Jackson, *U. S. Bureau of Standards Scientific Paper* 232, 1914.

though deficient in decolorizing power and unsuited for the clarification of dark-colored products for polariscopic readings, has certain advantages in that it does not precipitate reducing sugars from solution and does not form soluble lead-sugar compounds of different specific rotation. For these reasons the neutral acetate of lead should be employed for clarifying wherever possible in preference to the basic salt.

Neutral Lead Acetate Solution. In preparing the neutral acetate of lead reagent, a concentrated solution of commercial lead acetate (sugar of lead) is made, any free alkali or acid neutralized with acetic acid or sodium hydroxide, and the liquid diluted to a density of 30° Bé. (54.3° Brix or 1.2536 density $\frac{20°}{4°}$). The solution is filtered and kept in a stock bottle ready for use.

Lead Subacetate Solution. Upon digesting litharge with normal acetate of lead solution varying amounts of lead oxide are dissolved according to the time and temperature of digestion. Numerous methods are employed for preparing lead subacetate reagent. The following examples are given:

I. *Concentrated Solution.*[20] Heat, nearly to boiling, for about half an hour, 860 g. of neutral lead acetate, 260 g. of litharge, and 500 ml. of water. Add water to compensate for the loss by evaporation. Cool, settle, and decant the clear solution. The solution may be prepared without heat, provided that the mixture is set aside several hours with frequent shaking.

Dilute Solution. Proceed as described above, using, however, 1000 ml. of water. The solution should be diluted with cold, recently boiled distilled water to 54.3° Brix (30° Bé., or 1.2536 density $\frac{20°}{4°}$).

II.[21] Boil 430 g. of neutral lead acetate, 130 g. of litharge, and 1 liter of water for 30 minutes Allow the mixture to cool and settle and then dilute the supernatant liquid to 1.25 sp. gr. with recently boiled distilled water.

III. Lead subacetate solution may also be prepared by dissolving the solid basic salt (see p. 319). The concentrated solution is diluted with distilled water to a specific gravity of 1.25.

IV. The International Commission prescribes the use of lead subacetate solution prepared according to the German Pharmacopœia: 600 g. of neutral lead acetate and 200 g. of litharge (free from carbonate) are rubbed together with 100 ml. of distilled water. The mixture is heated on the water bath until it becomes white or light pink. Then 1900 ml. of water is stirred in gradually, and the mixture is allowed to settle in a covered vessel. The solution is decanted or filtered, and kept

[20] "Spencer's Handbook for Cane Sugar Manufacturers," 6th ed., p. 414.
[21] "Methods of Analysis, A. O. A. C.," 5th ed., p. 490, 1940.

in well-stoppered bottles. The subacetate solution must be strongly alkaline toward litmus, and have a specific gravity of 1.235 to 1.240. The concentrated mixture may also be prepared in the cold, by allowing it to stand for about a week with frequent shaking. Stock solutions of lead subacetate, both in bottle and burette, should be protected by a soda-lime tube from the carbon dioxide of the air to prevent deposition of lead carbonate (see Fig. 173).

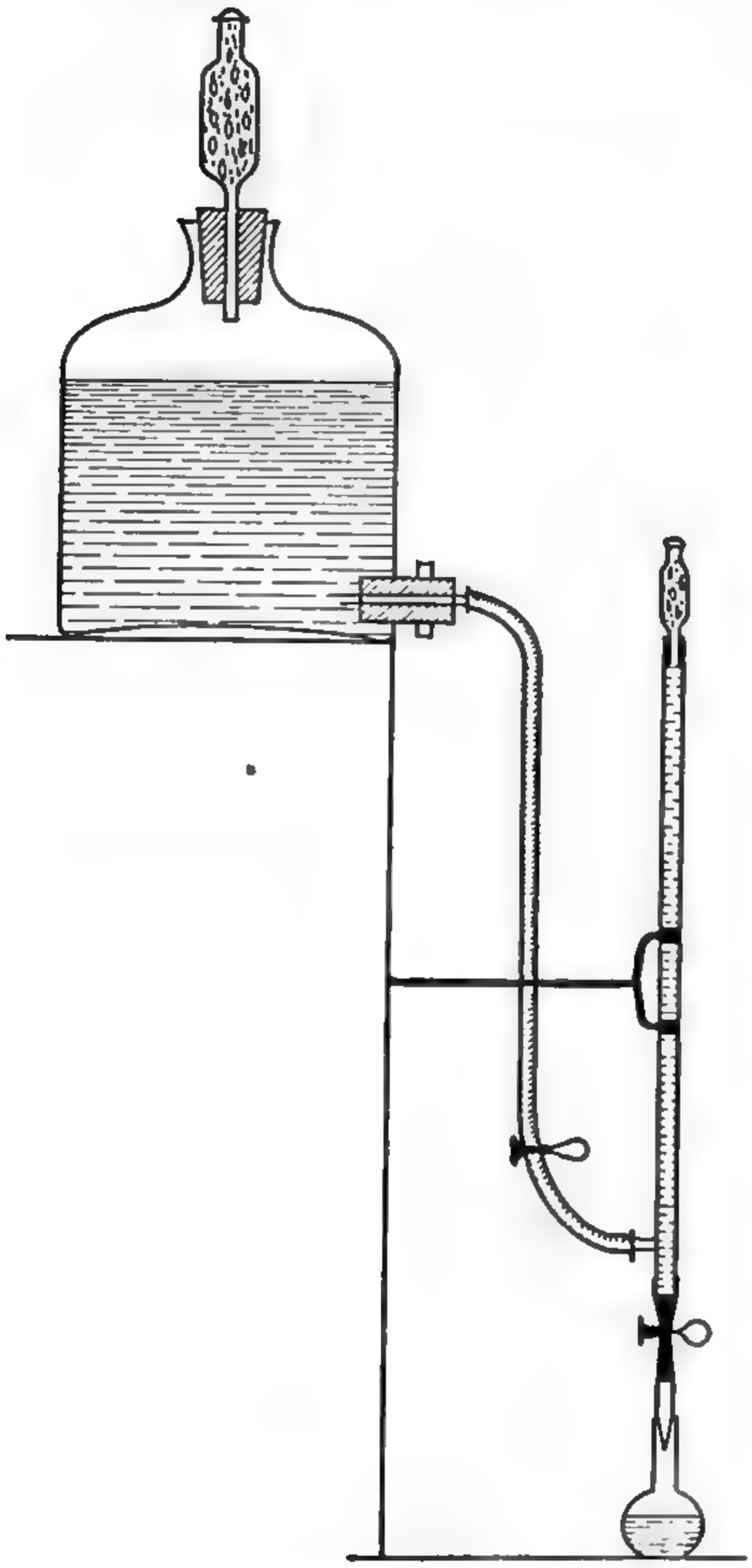


FIG. 173. Stock bottle and burette for lead subacetate solution.

The purifying and decolorizing effect of lead subacetate varies greatly with its composition, and especially with its basicity.[22] The official methods used in Czechoslovakia therefore prescribe that the lead subacetate solution must contain 30 ± 1 per cent of the total lead in the form of lead oxide (basic lead), and 70 ± 1 per cent combined with acetic acid (neutral lead). The composition of the reagent may be checked by the following method of Snell:[23]

Determination of Basic Lead. Pipette 10 ml. of the lead subacetate solution into a small beaker or Erlenmeyer flask. Add exactly 50 ml. of 0.5 *N* oxalic or sulfuric acid. Mix and allow to stand until the precipitate is well settled. Filter into a 250-ml. volumetric flask, washing the precipitate thoroughly with water. Make up to the mark, and titrate 50 ml. aliquots with 0.1 *N* sodium hydroxide, using phenolphthalein as indicator. Half the difference between 50 and the number of milliliters of 0.1 *N* sodium hydroxide used represents the number of milliliters of sulfuric acid neutralized by 1 ml. of the lead subacetate solution. This number, multiplied by 0.01036, gives basic lead in 1 ml. of the lead subacetate solution.

[22] Sommer, *Z. Zuckerind. čechoslovak. Rep.*, 53, 45 (1928/29).
[23] *J. Assoc. Official Agr. Chem.*, 4, 430 (1921).

Determination of Total Lead. Pipette 5 ml. of the lead subacetate solution into a 250-ml. flask. Add sufficient acetic acid (about 1 ml. of 30 per cent acid) to prevent precipitation on diluting, and make up to the mark. Treat 25-ml. aliquots with 25 ml. of water and dilute sulfuric acid in slight excess (about 1 ml. of 5 *N* acid). Mix, add 10 ml. of 95 per cent alcohol, and allow to stand for 3 hours or more. Filter on a tared Gooch crucible, wash with alcohol, dry in an oven, and ignite to bright redness in the muffle. Cool and weigh. Multiply the weight of the precipitate by 1.3665 (2 Pb ÷ $PbSO_4$) to obtain the weight of lead in 1 ml. of solution.

The difference between the total lead and the basic lead gives the neutral lead.

If desired, the acetic acid in the subacetate can be determined by distillation with phosphoric acid.[24]

I. Errors of Clarification Due to Volume of Precipitated Impurities

Since all sugar solutions after clarification with lead subacetate, or other means, are made up to a definite volume, the space occupied by the precipitated impurities will cause the sugar solution to occupy a somewhat smaller volume than that of the flask in which the solution was made up. An increase in concentration and also in polarization is the result.

Scheibler's Method of Double Dilution. Several methods have been devised for estimating the extent of this error. The first to be described is Scheibler's[25] method of double dilution. In this method a normal weight of product is dissolved in water, clarified with a measured volume of lead subacetate, the volume completed, the solution filtered, and read in the usual way. A second normal weight of product is then weighed out, clarified with the same volume of reagent as before, and the solution made up to twice the volume of the previous experiment. The second solution is filtered and polarized as before. The true polarization (P) is then calculated as follows:

Let P_1 be the polarization of the first solution made up to volume V, and P_2 the polarization of the second solution made up to volume $2V$. Let v be the volume of the precipitated impurities, which is assumed to be the same in both experiments. The normal weight in the second solution may be considered to be divided as follows: one half dissolved in volume V free from precipitate, the reading of which

[24] *Z. Zuckerind. čechoslovak. Rep.*, **52**, 644 (1927/28).
[25] *Z. Ver. deut. Zucker-Ind.*, **25**, 1054 (1875).

would be $P/2$, and one half dissolved in volume V containing precipitate, the reading of which would be $P_1/2$. The sum of these quantities divided by 2 is the value of P_2, or

$$\frac{\frac{P}{2}+\frac{P_1}{2}}{2}=P_2$$

whence $P = 4P_2 - P_1$. In other words the true polarization is equal to four times the polarization of the diluted solution less the polarization of the undiluted solution.[26]

Example. Polarization of 26 g. raw sugar, dissolved in water, clarified with 2 ml. lead subacetate and made to 100 ml. = 94.2 (P_1).

Polarization of 26 g. same sugar, dissolved in water, clarified with 2 ml. lead subacetate and made to 200 ml. = 47.0 (P_2).

True polarization (P) = $(47.0 \times 4) - 94.2 = 93.8$.

The volume v occupied by the precipitated impurities is calculated as follows. The reading P_1 of the undiluted solution is equal to $\frac{V \times P}{V - v}$, whence $v = \frac{V(P_1 - P)}{P_1}$.

Example. Required the volume of the lead precipitate in the previous example.

Substituting the values for V, P, and P_1, we obtain

$$v = 100\,\frac{(94.2 - 93.8)}{94.2} = 0.42 \text{ ml.}$$

Davoll[27] has shown that similarly $P_2 = \frac{V \times P}{2V - v}$. If the two equations for P_1 and P_2 are solved for P, then

$$P = \frac{P_1 \times P_2}{P_1 - P_2}$$

which formula gives a result for P slightly different from Scheibler's formula above. If, for example, $P = 50$, and $v = 2$, then $P_1 = 51.0204$

[26] The true polarization is also expressed in other ways as: multiply reading of dilute solution by 2, subtract the product from reading of undiluted solution, and twice the remainder subtracted from the reading of the undiluted solution will give the true polarization; or the difference between the reading of the undiluted solution, and twice the reading of diluted solution subtracted from twice the reading of the diluted solution, will give the true polarization.

[27] Private communication.

and $P_2 = 25.2525$. With Davoll's formula the result for P is exactly 50, but with Scheibler's formula it is 49.99, a trifle low.

The method of Scheibler owing to its rapidity and ease of execution has been very widely used for correcting polarizations for the error due to volume of the lead precipitate. The method is open to several objections. It is not probable that the volume of the precipitate is exactly the same in the diluted as in the undiluted solution, but the principal objection against the method is the very large multiplication of any error made in reading the diluted solution.

Sachs's Method of Correcting Precipitate Error. The method devised by Sachs[28] in 1880 for determining the error due to volume of precipitate was intended to obviate the errors of Scheibler's method. In the Sachs method the precipitate of impurities obtained in the clarification of the sugar solution is washed with cold and hot water until all sugar is removed. The precipitate is then transferred to a 100-ml. flask, a one-half normal weight of sucrose added, the latter dissolved and the volume completed to 100 ml. The solution is mixed, filtered, and polarized in a 400-mm. tube. The volume of precipitate is then calculated as follows: Let P = the true polarization of the sucrose used and P_1 = the polarization of the sucrose with precipitate. The volume (v) of precipitate is then found by the equation

$$v = \frac{100\,(P_1 - P)}{P_1}$$

Example. A normal weight of granulated sugar dissolved to 100 ml. polarized 99.8 in a 200-mm. tube.

A one-half normal weight of the same sugar + lead precipitate dissolved to 100 ml. polarized 100.25 in a 400-mm. tube. Volume of precipitate (v) = $100\,\frac{(100.25 - 99.8)}{100.25} = 0.45$ ml.

The volume (v) of lead precipitate being known, the true polarization (P) of a product may be determined by the equation $P = \frac{VP_1 - vP_1}{V}$, or, when $V = 100$, $P = \frac{100\,P_1 - vP_1}{100}$.

Example. The polarization of a raw sugar (26 g. to 100 ml.) was 96.20 (P_1). The volume of the lead precipitate by Sachs's method was 0.22 ml. (v).

The true polarization (P) of the sugar = $\frac{100 \times 96.2 - 0.22 \times 96.2}{100} = 95.99$.

The method of Sachs has been modified as follows. Instead of

[28] *Z. Ver. deut. Zucker-Ind.*, 30, 229 (1880).

making a polarization with the washed precipitate the latter is first dried. From the weight and specific gravity of the dried lead precipitate the volume is calculated $\left(v = \frac{w}{\text{sp. gr.}}\right)$, and from the volume the true polarization is determined by means of the preceding formula.

The specific gravity of the dried lead precipitates of raw cane sugars was determined by Wiechmann[29] by weighing in a pycnometer with benzine. The results of Wiechmann are given in Table LII.

TABLE LII

SPECIFIC GRAVITY AND VOLUME OF LEAD PRECIPITATES FROM 26 G. OF DIFFERENT RAW CANE SUGARS

Sugar	Weight of Precipitate in Grams	Specific Gravity $H_2O = 1.00$	Volume in ml.
Jamaica muscovado	0.4559	1.88	0.24
Maceio muscovado	0.8112	1.65	0.49
San Domingo centrifugal	0.2525	2.91	0.09
Sandwich Island centrifugal	0.1378	2.84	0.05
San Domingo concrete	1.0139	3.80	0.27
Puerto Rico molasses sugar	0.8959	4.35	0.21
Sandwich Islands	1.0195	4.38	0.23
Cebu mats	1.5400	2.17	0.71
Manila mats	1.3350	2.22	0.60

Similar results by Horne are given in Table LIII. The method employed by Horne[30] consists in weighing the freshly washed precipitate in a calibrated pycnometer filled to the mark with distilled water; the precipitate is then washed upon a weighed filter, dried, and weighed.

In later investigations by Browne and Wiley[31] the average specific gravity of the dried lead precipitate from three Cuban centrifugal sugars was found to be 2.47, and for four Philippine mat sugars, 2.74. The PbO content of the precipitates was 46.85 and 49.56 per cent respectively. From these figures it is calculated that the volume error caused by the lead precipitate is 0.10 to 0.12 ml. for the Cuban, and 0.32 to 0.48 ml. for the Philippine sugars, if a normal weight is dissolved to 100 ml.

It is very doubtful whether the lead precipitate, after drying or even after washing with water, has the same specific gravity as it has when freshly obtained in the sugar solution. Colloid chemical considerations make it very probable that the precipitate in the solution contains

[29] *Proc. Fifth Int. Congr. Applied Chem.* (Berlin, 1904), III, 118.
[30] *J. Am. Chem. Soc.*, 26, 186 (1904).
[31] *Facts About Sugar*, 12, 371 (1921).

absorbed water, and that therefore the volume error is really greater than would appear from the figures given above. Nevertheless, the methods which are based upon the separation and examination of the washed lead precipitate throw much light upon the errors of clarification; they are not adapted to practical work, however, owing to the large amount of time and labor involved.

Horne's Method of Dry Defecation. A third method of eliminating the volume of precipitate error is Horne's[32] process of dry defecation. The method is thus described by its author:

The normal weight of sugar is dissolved in water in a 100-ml. flask and made up to the mark without defecation. The concentration is thus at exactly the proper degree. It now remains to defecate the solution properly by precipitating the impurities in such a way as to produce the minimum change in the concentration of the solution of sucrose. This is accomplished by adding to the 100 ml. of liquid small quantities of powdered anhydrous lead subacetate until the impurities are nearly all precipitated. This point is as easily determined as in the defecation by a solution of the same salt. The organic and mineral-acid radicals in the solution combine with and precipitate the lead and lead oxide of the dry salt, while the acetic-acid radical of the lead subacetate passes into solution to combine with the bases originally united to the other acid radicals.

Results obtained by Horne upon 12 raw cane sugars are given in Table LIII; they show a very close agreement between the corrected polarization by Sachs's method and the polarization by dry defecation.

TABLE LIII

RESULTS OBTAINED BY HORNE'S METHOD OF DRY DEFECATION

	Grade, Country	Ordinary Polarization	Specific Gravity of Precipitate	Volume of Precipitate	Corrected Polarization	Dry Lead Polarization
				ml.		
1	Centrifugal	95.0	2.98	0.10	94.9	94.9
2	Centrifugal (mixed samples)	94.5	....	0.0765	94.43	94.4
3	Centrifugal, Trinidad	96.95	2.91	0.0378	96.91	96.95
4	Centrifugal, Java	97.425	2.30	0.0884	97.33	97.375
5	Muscovado, St. Croix	85.8	1.91	0.4118	85.45	85.5
6	Molasses, Cuba	89.4	3.20	0.39	89.05	89.0
7	Molasses	89.225	2.85	0.4204	88.85	88.85
8	Molasses	86.45	1.96	0.7108	85.84	85.95
9	Molasses	90.675	3.20	0.3204	90.39	90.45
10	Molasses	89.35	....	0.8500	88.59	88.775
11	Molasses	89.4	3.01	0.4554	88.99	89.0
12	Molasses, Cuba	88.4	2.64	0.4924	87.97	88.0

[32] *J. Am. Chem. Soc.*, 26, 186 (1904).

Horne's method has been tested by a number of chemists upon raw cane sugars with results very similar to the above. Pellet,[33] however, has criticized the method principally upon the ground that the increase in polarization due to the volume of precipitate is not as great as calculated, owing to the decrease in polarization caused by the retention of sucrose in the precipitate, this retention error frequently more than counterbalancing the error due to volume of precipitate. Subsequent results by Horne[34] and other chemists show, however, that there is no appreciable retention of sucrose when the dry lead reagent is used in minimum amounts. Another objection by Pellet, that only part of the lead salt acts and that the rest passes into solution, thus increasing the volume and diminishing the polarization, deserves consideration.

With the higher grade of sugar-house products there is no difficulty in securing a satisfactory clarification with a minimum amount of the dry lead salt, the lead dissolved being immediately precipitated and but very little remaining in solution. With low-grade sugars, molasses, etc., the case is otherwise. If dry lead subacetate, or subacetate solution, is added to a solution of such products to the point of satisfactory clarification a considerable amount of lead salt will usually remain dissolved. The rule of adding the powdered salt until no more precipitate forms is not always a criterion of the absence of lead in the filtrate. When subacetate is added to solutions of low purity the first portions of lead are completely precipitated; then comes a point where with the formation of additional precipitate a small amount of lead remains in solution; the amount of the latter continues to increase until at the point where no more precipitate is formed nearly all the lead added remains dissolved. (See Table LIV.) With very low-grade products there is therefore a danger that the dry lead salt will increase the volume of solution; whether this increase will cause a lowering of the polarization or not will depend upon the character of the product. With low-grade sugar-cane products the error due to increase in volume of solution may be more than counterbalanced by the precipitation of levorotatory fructose.

When increasing quantities of lead subacetate are added to the solution of a sugar product, the color of the filtrate becomes progressively lighter, but after a certain point is reached it darkens again owing to increased alkalinity.

In the following experiments by Hall[35] in the New York Sugar Trade Laboratory the effect of increasing amounts of dry lead subacetate upon

[33] *Bull. assoc. chim. sucr. dist.*, **23**, 285 (1905/06).
[34] *J. Am. Chem. Soc.*, **29**, 926 (1907).
[35] *Bull.* 122, U. S. Bur. Chem., p. 225.

the polarization of a Philippine mat sugar was studied. The quantity of lead in the clarified filtrates was determined and the dilution calculated by allowing an increase of 0.22 ml. in volume for 1 g. of dry subacetate dissolved in 100 ml. of solution.

TABLE LIV

ESTIMATED DILUTION OF A SUGAR SOLUTION BY DRY LEAD SUBACETATE

Clarifying Agent	Amount of Clarifying Agent Used	In 100 ml. Filtrate		Estimated Dilution	Polarization
		PbO	Lead Subacetate		
		grams	grams	ml.	
Subacetate solution.	3.0 ml.	0.2678	.....	....	86.70
Dry subacetate.....	0.5 g.	Trace	.....	Trace	Too dark to read
Dry subacetate.....	1.0 g.	0.1530	(0.20)	0.05	86.50
Dry subacetate.....	2.0 g.	0.7203	(0.94)	0.20	86.60
Dry subacetate.....	4.0 g.	2.1078	(2.73)	0.60	86.50

It is noted that with an estimated dilution of 0.2 ml. instead of a decrease in polarization, as would be expected, there is an increase. With an estimated dilution of 0.6 ml. the reading is the same as that first obtained, so that the combined effect of the dry lead upon the precipitation of fructose and upon the lowering of the rotation of the fructose in solution is seen to be most pronounced. With sugar-cane products the use of dry lead subacetate to the point of satisfactory clarification would seem to involve no decrease in polarization. With low-grade sugar-beet and other products, which are comparatively free from fructose, however, there is a danger of too low polarization since there is no compensating influence for the dilution caused by the excess of lead subacetate dissolved.

De Wolff[36] has concluded from carefully conducted experiments that in the polarization of raw sugars the volume error is completely corrected for by the addition of dry lead subacetate 15 per cent in excess of that required for clarification. If a larger excess is used the volume of the solution increases, but the resulting minus error is at least partly compensated for by the plus error due to the effect of lead subacetate on fructose and amino compounds discussed below.

In using dry lead subacetate for defecation the chemist must be certain of the composition of his preparation. The powdered salt must be dry and should contain the requisite amount of basic lead. Some samples of dry lead subacetate sold by the trade have been found to consist almost entirely of the normal acetate. A very pure anhy-

[36] *Chem. Weekblad*, 31, 475, 655 (1934).

drous lead subacetate is manufactured having closely the formula, $3\,Pb(C_2H_3O_2)_2 \cdot 2\,PbO$.[37] A sample of such a preparation analyzed at the New York Sugar Trade Laboratory gave the following results:

	Total Pb	Basic Pb
	per cent	per cent
Found	73.00	30.03
Theory for $3\,Pb(C_2H_3O_2)_2 \cdot 2\,PbO$	72.84	29.14

The above formula would correspond to a mixture of 4 parts of the basic acetate $3\,Pb(C_2H_3O_2)_2 \cdot PbO$ and 3 parts of the basic acetate $Pb(C_2H_3O_2)_2 \cdot 2\,PbO$.[38]

A solution of lead subacetate of 1.259 sp. gr., as employed for clarification in the wet way, was found to contain 0.2426 g. total Pb per 1 ml. One-third gram dry salt is therefore equivalent to 1 ml. subacetate solution in clarifying power. A low-grade sugar requiring 6 ml. of subacetate solution of the above strength for clarification would accordingly need 2 g. of salt for dry defecation.

The Sugar Institute of Czechoslovakia prescribes the following specifications for dry lead subacetate:[39] Moisture not to exceed 2 per cent; weight per unit volume of the salt not to exceed 1.4; basic lead content from 30.2 to 31.7 per cent; decolorizing effect of 0.35 g. on normal weight of standard beet sugar not less than 70 per cent.

The dry subacetate of lead employed in sugar analysis should be finely ground in order that it may be acted upon quickly and completely by the dissolved impurities. The tendency to form insoluble crusts upon the powdered grains of dry salt has been noted by Horne, especially in refinery products subjected to the influence of bone black. In such cases Horne recommends the addition of a little dry sand with the powdered lead salt; the particles of sand in shaking will grind off the crusts of insoluble matter and allow the lead to be acted upon.

Dry lead subacetate offers decided advantages for routine factory control. In the polarization of juices the Schmitz method (p. 309) can be further simplified. The proper amount of the salt is added to any desired volume of juice, no weighing or accurate measuring being required. If the reading of the clarified filtrate is R, then the polarization

[37] Dry lead subacetate according to Horne's specifications is manufactured exclusively by the Baker and Adamson Division of the General Chemical Co.

[38] Jackson in an unpublished experiment communicated to the authors shows that Horne's dry subacetate is in fact a mixture of these two basic acetates.

[39] Šandera, *Z. Zuckerind. čechoslovak. Rep.*, **59**, 177 (1934/35).

P, on the normal-weight basis, is found by the formula

$$P = \frac{26\,R}{99.718 \times \text{sp. gr.}\,{}^{20^\circ}_{20^\circ}\text{ of juice}}$$

Horne has calculated tables showing the values of P for varying degrees Brix and varying values of R, and these tables are found in the usual textbooks of sugar factory control.

If the normal-weight method is used in connection with dry lead clarification, only one weighing need be made, and the solution will serve not only for the determination of the polarization, but without clarification also for that of Brix by refractometer, *p*H, color, turbidity, conductivity-ash, lime salts, acidity or alkalinity, etc.[40]

II. Errors of Clarification Due to Precipitation of Sugars from Solution

In the absence of free alkalies sucrose is not precipitated from solution by lead subacetate. Reducing sugars, however, are precipitated by solutions of basic lead salts. This precipitation does not occur with the amounts of lead used in ordinary clarification except in the presence of those salts or acids which form insoluble lead compounds[41] (as chlorides, sulfates, phosphates, carbonates, oxalates, tartrates, malates, etc.). Whether this precipitation of reducing sugars is due to simple occlusion or to the formation of insoluble sugar-lead complexes is not definitely known. Deerr[42] has found that the precipitate produced by basic lead in the presence of sodium sulfate can be readily broken up quantitatively by digestion with dilute sulfuric acid, but not by treatment with sodium sulfate solution. This indicates that the reducing sugars are in chemical combination with the lead.

The extent to which the common reducing sugars glucose and fructose are precipitated by different lead clarifying agents has been investigated by Bryan.[43] Separate solutions of glucose and fructose were prepared, using 5 g. of sugar with 1 g. each of magnesium sulfate and ammonium tartrate. To 50 ml. of this solution the clarifying agent was added and the volume made up to 100 ml. After filtering, the excess of lead was removed with potassium oxalate, and the sugar in solution de-

[40] Vondrák, *Z. Zuckerind. čechoslovak. Rep.*, **52**, 381 (1927/28); Konn, *ibid.*, **54**, 326 (1929/30); Dolínek, *ibid.*, **55**, 439 (1930/31); Bachler, *Facts About Sugar*, **28**, 420 (1933).

[41] Prinsen Geerligs, *Deut. Zuckerind.*, **23**, 1753 (1898).

[42] *Intern. Sugar J.*, **18**, 402 (1916).

[43] *Bull.* **116**, U. S. Bur. Chem., p. 73.

termined by Allihn's method. The results of Bryan's experiments are given in the following table.

TABLE LV

PRECIPITATION OF GLUCOSE AND FRUCTOSE BY BASIC LEAD SALTS

Clarifying Agent	Amount per 100 ml. of Solution	Glucose Precipitated	Fructose Precipitated
		per cent of total	per cent of total
Neutral lead acetate solution....	3.5 ml.	0.93	0.00
Neutral lead acetate solution.....	7.0 ml.	0.84	0.00
Lead subacetate solution.....	3.5 ml.	3.35	8.03
Lead subacetate solution.....	7.0 ml.	8.34	19.91
Dry lead subacetate.....	1.0 g.	3.85	14.93
Dry lead subacetate.....	2.5 g.	17.48	35.33
Basic lead nitrate solution.....	4.0 ml.	6.27	13.84
Basic lead nitrate solution.....	8.0 ml.	5.61	25.12

It is seen that neutral lead acetate precipitates but very little reducing sugar. Eynon and Lane claim[44] that neither glucose nor fructose is precipitated at all by neutral lead acetate followed by potassium oxalate, but Meade states[45] that the effect of neutral lead acetate on reducing sugars is appreciable. This question is further discussed in Chapter XIV.

The basic lead salts remove a large percentage of both glucose and fructose, the fructose, however, in more than double the amount. This precipitation of reducing sugars during clarification has a most marked effect upon the polarization, the removal of glucose from solution diminishing the dextrorotation, and that of fructose the levorotation. The greater precipitation of fructose in mixtures with sucrose and glucose, as in the clarification of sugar-cane products, jellies, jams, etc., causes an increase in the dextrorotation, frequently exceeding 1° V. The precipitation of reducing sugars, though of no consequence as regards the saccharimetric or gravimetric determination of sucrose, is of the greatest importance when the valuation of a product is based upon the polarization alone, or upon a determination of reducing sugars. The precipitation of sugars increases with the alkalinity of the clarifying agent, but an excess of the latter may redissolve the precipitate.

[44] *J. Soc. Chem. Ind.*, 42, 463 (1923).

[45] "Spencer's Handbook," 7th ed. by Meade, p. 239, 1929.

III. Errors of Clarification Due to Changes in Specific Rotation

Action of Lead Subacetate on Rotation of Sucrose. The results of Müntz,[46] Weisberg,[47] Svoboda,[48] Gröger,[49] and other investigators show no perceptible influence of basic lead acetate upon the specific rotation of sucrose in aqueous solution. Experiments by Bates and Blake[50] indicate, however, a very perceptible influence if the lead reagent is used in large excess. Table LVI, showing the loss and gain in polarization for a normal weight of pure sucrose, is taken from the work of Bates and Blake.

TABLE LVI

Number of Milliliters of Basic Lead Solution (1.25 sp. gr.) Added	Difference in Degrees Ventzke between Similar Solutions, One with the Other without Basic Lead Acetate	Number of Milliliters of Basic Lead Solution (1.25 sp. gr.) Added	Difference in Degrees Ventzke between Similar Solutions, One with the Other without Basic Lead Acetate
0.5	−0.09	10.0	+0.19
1.0	−0.13	15.0	+0.29
2.0	−0.13	20.0	+0.45
3.0	−0.08	25.0	+0.58
4.0	−0.06	30.0	+0.62
5.0	−0.03	35.0	+0.77
6.0	0.00	40.0	+0.77
7.0	+0.05	63.0	+0.95
8.0	+0.09		

The + sign indicates that the solution containing the lead subacetate gives the higher polarization, and conversely for the — sign.

Action of Lead Subacetate on Rotation of Fructose. While the specific rotation of sucrose under the ordinary conditions of analysis is not modified sufficiently by subacetate of lead to introduce serious errors, the case is otherwise with fructose. Gill[51] first showed, in 1871, that the specific rotation of fructose was greatly diminished by the presence of lead subacetate, this decrease being so great that in the presence of sufficient basic lead the rotation of invert sugar ($[\alpha]_D^{20} = -20$) was changed to the right. This change in rotation is due to the formation of soluble dextrorotatory lead fructosate, the presence of which, even in small amounts, is sufficient to reduce the figure for the ro-

[46] *J. fabr. sucre*, 17, 25 (1876).
[47] *Sucr. belge*, 16, 407 (1888).
[48] *Z. Ver. deut. Zucker-Ind.*, 46, 107 (1896).
[49] *Oesterr.-ungar. Z. Zuckerind. Landw.*, 30, 429 (1907).
[50] *Bull. U. S. Bur. Standards*, 3 (1), 105 (1907).
[51] *Z. Ver. deut. Zucker-Ind.*, 21, 257 (1871).

tation of fructose ($[\alpha]_D^{20} = -92$) below that of glucose ($[\alpha]_D = +52.5$). Gill[52] showed that the error due to formation of soluble lead fructosate could be entirely avoided by adding acetic acid to the point of acidity, thus decomposing the soluble lead fructosate into lead acetate and free fructose of normal specific rotation. If the soluble lead fructosate is not decomposed by some precipitating agent of lead, acetic acid should be added to weak acidity before the volume of the clarified solution is made up to 100 ml. for the direct polarization of low-grade fructose-containing products.

Action of Lead Subacetate on Rotation of Amino Compounds. Both cane and beet products contain small quantities of optically active amino acids and amides. The principal ones are *l*-aspartic acid (monoamino succinic acid), *l*-asparagine (monoamide of *l*-aspartic acid), *d*-glutaminic acid (monoamino glutaric acid), and *d*-glutamine (monoamide of *d*-glutaminic acid). Cane products contain mostly asparagine and aspartic acid.[53] In beet products glutamine and glutaminic acid usually predominate,[54] but in certain countries and at certain seasons they may be entirely absent, being replaced by asparagine and aspartic acid.[55]

l-Asparagine is slightly levoratatory, $\alpha_D^{20} = -5.5$ to -8, depending on the concentration; *l*-aspartic acid has $\alpha_D^{20} = +4.4$. But when lead subacetate is added to solutions of either asparagine or aspartic acid they become strongly dextrorotatory, and the rotation may equal that of sucrose. *d*-Glutamine is dextrorotatory, $\alpha_D^{20} = +7$ to 10, depending on the concentration; the specific rotation of *d*-glutaminic acid is of about the same order as that of *d*-glutamine. Both become levorotatory upon the addition of lead subacetate, the specific rotation falling to -9 to -21, according to concentration.

It is evident from the figures given above that clarification with lead subacetate raises the polarization of cane or beet products in which asparagine and aspartic acid predominate. In experiments made at the New York Sugar Trade Laboratory it was found that when 0.1 per cent aspartic acid, neutralized to *p*H 7 with sodium hydroxide, is added to a mixture containing 96 per cent sucrose, 0.5 per cent each of glucose and fructose, and 0.25 per cent each of potassium sulfate and potassium aconitate, the normal weight of the mixture, after clarifica-

[52] *Loc. cit.* See also "Spencer's Handbook for Cane Sugar Manufacturers," 7th ed., p. 226; Edson, *Z. Ver. Deut. Zucker-Ind.*, 40, 1037 (1890); Pellet, *Bull. assoc. chim. sucr. dist.*, 14, 28, 141 (1896/97).

[53] Zerban, *Proc. Eighth Int. Congr. Appl. Chem.*, 8, 103 (1912).

[54] Sellier, *Bull. assoc. chim. sucr. dist.*, 27, 190 (1909/10).

[55] Smoleński, *Z. Ver. deut. Zucker-Ind.*, 62, 791 (1912).

tion with 1 ml. lead subacetate solution, polarizes 0.23° V. higher than when the aspartic acid is absent. Two milliliters of lead subacetate solution raised the polarization 0.38°.

Sommer[56] has observed that, as the PbO content of the lead subacetate solution is increased, the polarization of beet molasses clarified with it becomes lower and lower; he has explained this result by the presence of salts of glutaminic acid in the molasses.

Correction for the Combined Lead Errors. A graphical method to correct for the errors in polarization caused by the use of lead subacetate was first proposed by Jackson and has been further elaborated by Guézé.[57] Increasing amounts of lead subacetate solution, of dry lead subacetate, and if possible of neutral lead acetate, are added to different portions of the same solution, the solutions are polarized, and the polarizations are plotted against the quantities of each lead salt. The curves obtained are extrapolated to zero quantity of each lead salt, and the polarization at this point gives the corrected polarization. The difficulty with this method is that the solutions become darker and darker as the quantity of clarifying agent is reduced, and the polarizations become more and more uncertain so that the exact trend of the curves near the point for zero quantity of clarifying agent cannot be found with any degree of exactness. Great caution must therefore be exercised in the extrapolation and interpretation of the curves. Furthermore, the procedure requires too much time to be applicable to routine polarizations.

Guézé has simplified his method for use with cane molasses, since he found that the curves for dry lead subacetate are nearly straight lines. If clarification of a solution containing 10 g. of molasses in 100 ml., with A grams of dry lead subacetate, gives polarization P', and clarification with 2 A grams gives polarization P'', then the corrected polarization

$$P = 2P' - P'' - \alpha$$

The value of α increases with the ratio between A and P'. If A is between 2 and 4 g., the error in the corrected polarization, calculated to a normal weight of 20 g., does not exceed the error in reading the polarizations. This result requires further confirmation before the method can be recommended.

[56] *Z. Zuckerind. čechoslovak. Rep.*, 53, 45 (1928/29).
[57] *Bull. assoc. chim. sucr. dist.*, 53, 116 (1936).

Miscellaneous Methods of Clarification

Numerous modifications of the lead process of clarification have been proposed as a means of reducing or eliminating the several sources of error just mentioned. Freshly precipitated lead carbonate, lead chloride, and lead nitrate have been employed as clarifying agents, but with only indifferent success. Two methods of lead clarification which have found considerable favor in Europe, however, should be mentioned in addition to the processes previously described. These are Zamaron's method by means of hypochlorite of lime and neutral lead acetate, and Herles's method by means of basic lead nitrate.

Zamaron's[58] Method of Clarification with Hypochlorite. In a large mortar, 625 g. of dry commercial bleaching powder are thoroughly ground up with 1000 ml. of water. The mass is squeezed out in a sack and the extract filtered through paper. The solution thus obtained (700 ml. to 800 ml. of about 18° Bé.), is preserved in a stoppered bottle of dark glass away from the light.

The solution to be clarified is treated with a few milliliters of the hypochlorite solution, sufficient to effect decolorization, and then a few milliliters of neutral lead acetate solution are added. There is usually a slight rise in temperature after addition of the clarifying agents so that the solution must be recooled before making to volume.

The Zamaron process secures usually a good clarification, does not precipitate reducing sugars, and forms no objectionable lead sugar compounds. The chief fault of the method is the volume of precipitate error, which in this case is augmented by the formation of considerable lead chloride.

According to Bhargava,[59] Zamaron's method causes inversion of sucrose. If sodium hypochlorite is substituted for bleaching powder there is no inversion, but the clarifying agents affect the rotation of sucrose.

Herles's[60] Method of Clarification with Basic Lead Nitrate. Dissolve 100 g. of solid sodium hydroxide in 2000 ml. of water; a second solution is prepared by dissolving 1000 g. of neutral lead nitrate in 2000 ml. of water. Upon mixing equal volumes of the two solutions basic lead nitrate is precipitated according to the equation

$$\underset{\text{Lead nitrate}}{2\ Pb(NO_3)_2} + 2\ NaOH \rightarrow \underset{\text{Basic lead nitrate}}{Pb(NO_3)_2 \cdot Pb(OH)_2} + 2\ NaNO_3$$

The precipitated basic lead nitrate is washed free from sodium com-

[58] Fribourg's "Analyse chimique," p. 129.
[59] *Intern. Sugar J.*, 31, 421 (1929).
[60] *Z. Zuckerind. Böhmen*, 13, 559 (1888/89); 14, 343 (1889/90); 21, 189 (1896/97).

pounds and then mixed with water to a cream, in which form it may be used for clarification.

The clarification is performed more commonly by forming the basic nitrate within the solution to be clarified. This is done by first adding a measured quantity of the lead nitrate solution (1 ml. to 15 ml. according to depth of color) and then, after mixing, an equal volume of the sodium hydroxide solution. After shaking, the solution is made to volume, well mixed, and filtered. Care must be taken that the reaction of the solution is not alkaline after mixing; this is best provided for by testing the two solutions against each other before using.

Formation of the basic lead nitrate within the solution gives usually a much better clarification than addition of the washed cream but has the disadvantage of introducing considerable sodium nitrate, which, if present in large quantity, will affect the rotation of the sugars.

The basic lead nitrate method gives an exceedingly brilliant clarification. The process is open, however, to the same errors as basic lead acetate. There is first the volume of precipitate error, which is further augmented by the copious bulk of the basic lead nitrate itself; and secondly there is a precipitation of reducing sugars as shown by the results of Bryan in Table LV.

Herles claimed[61] that the increase in polarization due to the volume of the precipitate is compensated for by the effect of the sodium nitrate formed during clarification on the rotation of the sucrose. As a matter of fact, in numerous analyses of beet molasses by Ofner,[62] the direct polarization after clarification with Herles's reagent was, during one campaign, almost always lower than that obtained with lead subacetate solution, while in another year it was often higher. Other investigators have made similar observations. Ofner found later[63] that the lower results are caused by too high alkalinity of the basic lead nitrate. The recipe for Herles's reagent was therefore changed in the official Czechoslovakian methods to read as follows:[64] 340 g. lead nitrate is dissolved in distilled water, transferred to a 1-liter flask, and made up to the mark. In another liter flask 32 g. sodium hydroxide (sticks) is dissolved and the solution made to the mark. For clarifying the normal weight of molasses, 20 ml. of each solution is used. According to Dorfmüller[65] reproducible results are obtained only if the two solutions are added in small successive portions and if the formation

[61] *Z. Zuckerind. čechoslovak. Rep.*, **47**, 188 (1922/23).
[62] *Z. Zuckerind. čechoslovak. Rep.*, **50**, 38, 505 (1925/26).
[63] *Z. Zuckerind. čechoslovak. Rep.*, **51**, 539 (1926/27).
[64] *Z. Zuckerind. čechoslovak. Rep.*, **53**, 53 (1928/29).
[65] *Z. Ver. deut. Zucker-Ind.*, **74**, 135 (1924).

of foam is carefully avoided. Molasses which are alkaline toward phenolphthalein must first be neutralized with acetic acid.

For the simple polarization of cane molasses which are usually quite acid, Kalshoven and Sijlmans[66] recommend a more alkaline reagent, consisting of 30 ml. lead nitrate solution (600 g. per liter) and 20 ml. sodium hydroxide solution (80 g. NaOH per liter), added to a solution of 35.816 g. molasses in 250 Mohr cc. The filtrate is still acid, and the direct polarization checks closely with that obtained after clarification with lead subacetate solution.

The numerous errors incident to the use of basic lead compounds in clarification have led chemists to seek other means of decolorizing solutions for polarization. It is impossible, as well as unnecessary, to take up all the processes which have been devised to accomplish this end. Two of these methods, however, should be described: (1) decolorization by means of bone black or other chars; (2) decolorization by means of hydrosulfites, sulfoxylates, etc.

Decolorization of Sugar Solutions by Means of Bone Black or Vegetable Carbons. The use of bone black as a decolorizing agent in sugar refineries is well known. The same substance in a more finely divided specially prepared form is employed at times as a decolorizer in sugar analysis.

Purification of Bone Black. If purified animal charcoal (preferably blood charcoal) has not been obtained from the dealer the chemist may purify the commercial product as follows: The char is finely ground in a mortar and then digested several hours in the cold with dilute hydrochloric acid. The acid is then decanted; the char is brought upon a filter and washed with distilled water until all traces of hydrochloric acid are removed. After drying in a hot-air oven, the char is heated to dull redness in a covered porcelain crucible, and then, after cooling sufficiently, placed while still warm in a dry, stoppered bottle.

Several methods are followed in the employment of animal charcoal for decolorizing. One very common practice is to make up the solution to volume and shake thoroughly with a small quantity of charcoal, using from 0.5 to 3 g. according to depth of color. The contents of the flask are then poured upon a dry filter and the filtrate taken for polarization.

Absorption Error of Bone Black. In the above method of decolorizing, a certain error is introduced owing to the absorption and retention of sugar by the char. Sugars differ markedly in the extent to which they are absorbed by animal charcoal. With the simple reducing sugars, glucose, fructose, etc., the error through absorption is

[66] *Arch. Suikerind.*, 29, 989 (1921).

so small as to be almost negligible, but with sucrose and other higher saccharides the absorption is so great that an error of several degrees Ventzke may be occasioned in the polarization.

One method of eliminating the error through absorption of sucrose consists in adding a correction previously established by experiment upon pure sugar solutions. If, for example, a sucrose solution polarizing 95.0° V. gives, after shaking 50 ml. with 2 g. of charcoal for 5 minutes, a polarization of only 94.7° V., then a correction of 0.3° V. must be added tc all polarizations of about 95° V. for sugars decolorized in the same way. A correction table is thus made for sugar solutions of different concentrations, but in applying these corrections care must be taken that the quality and quantity of the char are alike in both instances and that the time of shaking is always the same. With impure products of variable composition the employment of absorption factors is attended with considerable uncertainty.

Spencer[67] has recommended a different method of employing animal charcoal for the purpose of reducing the absorption error to a minimum. The process is thus described:

> Place a small quantity of bone black, about 3 g., in a small plain filter, selecting a rather slow filtering paper. Add a volume of the solution equal to that of the char, or just completely moisten the latter, and let this liquid filter off. After four or five similar filtrations, the filtrates from which are rejected, test the filtrates by a polariscopic observation and note whether the reading varies. Solutions must be protected from evaporation during the filtration. As soon as the reading is constant, showing no further absorption, record it as the required number.

The method just described, though it largely eliminates, does not completely remove, the errors of absorption, for while the retention of sucrose by the char rapidly diminishes with each successive portion of solution, it soon becomes only a gradually receding quantity. This is shown by the following experiments upon a sucrose solution polarizing 49.9° V.

Fraction of Filtrate	Polarization	Absorption Error
First running	48.9	1.0
Second running	49.4	0.5
Third running	49.75	0.15
Fourth running	49.80	0.10
Fifth running	49.80	0.10

With dark-colored solutions it also happens that, with each succeeding portion of the filtrate, the charcoal loses its absorptive power for

[67] "Spencer's Handbook for Cane Sugar Manufacturers," 6th ed., p. 183.

coloring matter as well as for sucrose, so that the final running least free from the error of absorption is too dark for satisfactory polarization.

The general consensus of opinion regarding animal charcoal in sugar analysis is that it should be used as a decolorizing agent only as a last resort. Its employment in the polarization of raw cane sugars has been condemned by the International Commission upon Unification of Methods.[68]

Vegetable decolorizing carbons have been recommended by various authors as substitutes for bone black, on account of their great decolorizing power. Šandera[69] found that the adsorption of sucrose is reduced by the simultaneous adsorption of surface-active non-sugars, and that the polarization may even be increased. Different carbons vary greatly in their effect on the polarization. If carbon is used at all for decolorization it is advisable to add increasing quantities to equal portions of the solution, to construct a curve showing the polarization changes, and to extrapolate to zero quantity of carbon.

It is always safest not to resort to the use of carbons but to increase the accuracy of the readings by means of stronger light sources and a larger half-shadow angle in the saccharimeter.

Decolorization of Sugar Solutions by Means of Hydrosulfites. Attempts have been made to employ various decolorizing agents for the purpose of avoiding the precipitate errors of basic lead salts and the absorption error of bone black. The most promising of the numerous substances which have been tried in this connection are the salts and derivatives of hydrosulfurous (hyposulfurous) acid.[70]

The employment of commercial hydrosulfite preparations, such as "Blankit" and "Redo," has been common in the sugar factory, where they have been used for bleaching dark-colored massecuites and also, in solution, as a wash for whitening sugars in the centrifugal. They have also been employed by unscrupulous manufacturers for bleaching low-grade molasses in the preparation of table sirups.

[68] See p. 304.

[69] *Z. Zuckerind. čechoslovak. Rep.*, **57**, 293 (1932/33).

[70] The dry sodium hydrosulfite is prepared by allowing zinc, sodium bisulfite, and sulfuric acid to react in the following molecular proportions:

$$2\,NaHSO_3 + Zn + H_2SO_4 = ZnS_2O_4 + Na_2SO_4 + 2\,H_2O$$

The zinc hydrosulfite is then decomposed with sodium carbonate,

$$ZnS_2O_4 + Na_2CO_3 = Na_2S_2O_4 + ZnCO_3$$

The sodium hydrosulfite is salted out from solution by means of sodium chloride and dehydrated by warming with strong alcohol. The compound is then dried in vacuo at 50° to 60° C.

For their use in sugar analysis the solution to be decolorized is treated with a few milliliters of alumina cream and a few crystals of sodium hydrosulfite (0.1 g. to 1.0 g., according to the depth of color); after mixing and dissolving, the volume is made up to the mark, and the solution filtered. The filtrate should be polarized immediately.

In many cases there is a rapid redarkening of solutions decolorized with hydrosulfites. Weisberg,[71] from his study of the action of hydrosulfites, concludes that the bleaching action is a double one: first, by means of the free sulfurous acid when decolorization is permanent; and second by means of the nascent hydrogen which is evolved, when there is a redarkening of the solution through oxidation. After-darkening may be prevented by the use of another hydrosulfite derivative, sodium sulfoxylate formaldehyde, sold commercially as "Rongalite." This, however, is much slower in its bleaching action than hydrosulfite and is not always an effective decolorizing agent.

A serious objection against hydrosulfite is its action upon the polarizing power of certain reducing sugars. Bryan[72] has found that the polarizing power of glucose was decidedly lowered after the addition of hydrosulfite, owing to the formation of a levorotatory oxysulfonate. Rongalite did not produce this effect. Neither Rongalite nor hydrosulfite caused any immediate change in the polarization of fructose or sucrose. Numerous cases of inversion of sucrose by the prolonged action of hydrosulfites have been reported, however, in the literature.

The experience of chemists, in the use of hydrosulfites as decolorizing agents for sugar analysis, has been upon the whole unfavorable. In many cases the decolorized solution becomes turbid through separation of sulfur, thus rendering polarization impossible. The bleaching action of hydrosulfite is also limited, and the substance has but little decolorizing effect upon caramel substances, which are among the chief causes of discoloration in sugar-house products.

Various oxidizing agents have also been recommended for decolorization, as chlorine, hydrogen peroxide, and ozone, but these have either been found not to be very effective or to attack the sugars and to cause changes in rotation.

Aluminum Hydroxide as a Clarifying Agent. A common preparation, used in connection with other clarifying agents, yet having but little decolorizing power in itself, is aluminum hydroxide, or, as it is more generally termed, "alumina cream." The original method of

[71] *Centr. Zuckerind.*, 15, 975 (1907).
[72] *Bull.* 116, U. S. Bur. Chem., p. 76.

preparing alumina cream, as prescribed by the Association of Official Agricultural Chemists, was as follows:[73]

Prepare a cold saturated solution of alum in water and divide into two unequal portions. Add a slight excess of ammonium hydroxide to the larger portion and then add by degrees the remaining alum solution until a faintly acid reaction is secured.

The reagent as above prepared consists of aluminum hydroxide suspended in a solution of ammonium and potassium sulfates. The salts have a certain advantage, when alumina cream is used as an adjunct with lead salts, in helping to precipitate any excess of lead from solution. In certain cases, however, the presence of ammonium and potassium sulfates is detrimental, so that for many purposes it is better to employ a salt-free cream. For this reason the directions of the Association of Official Agricultural Chemists have been changed to read as follows:[74]

Prepare a cold saturated solution of alum in water. Add strong ammonium hydroxide with constant stirring until the solution is alkaline to litmus, allow the precipitate to settle, and wash by decantation with water until the wash water gives only a slight test for sulfates with barium chloride solution. Pour off the excess of water and store the residual cream in a stoppered bottle.

The clarifying effect of alumina cream is chiefly mechanical; its action consists largely in carrying down finely suspended or colloidal impurities which would otherwise escape filtration. When used in connection with lead subacetate it promotes the coagulation of the precipitated impurities and renders filtration more perfect and rapid.

For the polarization of very high-grade sugars, sirups, honeys, etc., alumina cream is the only clarifying agent required. With all such materials only the salt-free reagent should be used. About 2 ml. of the cream is sufficient for clarification, and the volume of aluminum hydroxide in this amount is too insignificant to affect the polarization.

Concentrated solutions of alum or aluminum sulfate are sometimes used with lead subacetate for clarifying. The precipitate formed between the lead salt and alum helps to remove coloring matter, but the increase in precipitate and other errors tend to nullify any advantages of the method.

Deerr's method of clarification with equivalent quantities of aluminum sulfate and barium hydroxide (see p. 421), leaving no soluble salts in solution, may also be used to determine simple polarization. The

[73] " Methods of Analysis, A. O. A. C." *Bull.* 107 (revised), U. S. Bur. Chem., p. 40.
[74] " Methods of Analysis, A. O. A. C.," 5th ed., p. 490, 1940.

volume error can be determined by Sachs's method, and an average correction applied to each class of products. The method has not come into general use, however, because of the advantages of clarification with dry lead subacetate, which is now very generally used in cane-sugar factories.

Comparisons of Different Clarifying Agents

A few examples, taken from the reports of Referees upon Sugar for the Association of Official Agricultural Chemists, are given in order to show the probable error of different clarifying agents in polarization.

TABLE LVII

Polarization of Mixtures of Sucrose, Glucose, and Fructose with 0.5 g. Ammonium Oxalate and 0.5 g. Sodium Sulfate, Using Different Clarifying Agents (Bryan)[75]

Clarifying Agent	Amount of Clarifying Agent Used	Direct Polarization, °V.
Alumina cream	5 ml.	89.00
Lead subacetate solution	3.5 ml.	89.50
Lead subacetate solution	7 ml.	89.55
Neutral lead acetate solution	3 ml.	89.20
Neutral lead acetate solution	6 ml.	89.20
Basic lead nitrate solution	4 ml.	89.00
Dry lead subacetate	1.5 g.	89.05
Sodium hydrosulfite	1 g.	88.60

Taking the experiment with alumina cream as the true polarization, it is seen that the lead subacetate solution gives a reading 0.5° V. too high and the neutral lead acetate 0.2° V. too high. The excess reading in the second case is due to the volume of precipitate and in the former to both volume of precipitate and precipitation of fructose. The dry lead subacetate and basic lead nitrate clarifications give readings practically identical with the true polarization. This might seem to indicate no precipitation of optically active reducing sugars; such a precipitation does take place, however, and the experiment only shows that in this particular instance the various errors of clarification happen to neutralize one another. Treatment with hydrosulfite gives a polarization below the true value owing to the change in rotation of the glucose.

The experiments in Table LVIII show a lower polarization using hydrosulfite, a result due in large part to the change in rotation of glucose. Basic lead acetate and nitrate solutions give much higher

[75] *Bull.* 116, U. S. Bur. Chem., p. 71.

TABLE LVIII

POLARIZATIONS OF RAW CANE SUGAR AND CANE MOLASSES, USING DIFFERENT CLARIFYING AGENTS (AVERAGE RESULTS OF SEVERAL COLLABORATORS)

Clarifying Agent	Direct Polarization	
	Sugar	Molasses
Alumina cream and hydrosulfite	+92.75	+41.99
Neutral lead acetate solution	92.92	42.46
Basic lead acetate solution	93.05	42.82
Basic lead nitrate solution	92.98	43.23
Dry lead subacetate	92.90	42.63

polarizations owing to both the volume of precipitate error and the precipitation of fructose. Neutral lead acetate solution and dry lead subacetate give polarizations between these two extremes, there being, however, in case of the former, a volume of precipitate error and in case of the dry lead an error due to precipitation of reducing sugars. The true polarization would be somewhere between the results obtained with hydrosulfite and neutral lead acetate.

The errors arising from the use of lead subacetate, dry or in solution, have been studied at the New York Sugar Trade Laboratory[76] in two different ways.

In the first series of tests 1485 raw-sugar samples, from the various sources supplying the American market, were clarified with the required quantity of lead subacetate solution, and another normal-weight solution with the corresponding quantity of dry lead subacetate. All polarizations were made on duplicate samples, by both methods, and the readings were taken at 20° C. on instruments with the Herzfeld-Schönrock scale. The results are shown in the following table.

Origin of Samples	No. of Samples	Av. Pol. Lead Soln.	Av. Pol. Dry Lead	Lead Volume Error
Cuba	505	96.7853	96.6209	0.1644
Puerto Rico	291	96.9818	96.8493	0.1325
Philippines	596	96.9570	96.8322	0.1248
Hawaii	59	97.8941	97.7932	0.1009
Louisiana and Florida	34	97.4059	97.3030	0.1029
	1485	96.9510	96.8126	0.1384

The average volume error ranged from 0.10 for Louisiana, Florida, and Hawaiian sugars to 0.16 for Cuban sugars, the general average for

[76] *J. Assoc. Official Agr. Chem.*, 18, 178 (1935); *Facts About Sugar*, 29, 277 (1934).

all samples being 0.14. Honig and Rooseboom[77] have reported an average volume error of 0.1° V. for Java raw sugars; in Czechoslovakian beet sugars the error amounts to 0.08° V., according to Šandera.[78] The general average, rounded off to the first decimal place, is 0.1° V.

In the second series of tests at the New York Sugar Trade Laboratory 91 raw sugars from various countries were used. Ten times the normal weight of each sugar was dissolved to 500 ml.; the solution was decolorized with the minimum quantity of Suchar and filtered. The treatment with decolorizing carbon removes all the suspended matter and also some of the dissolved impurities, and it follows from this that the volume error as well as that due to precipitation and rotation effects should be greater in the actual raw sugar than after treatment with activated carbon. Nevertheless, the decolorized filtrate may still be taken as representing a raw sugar, and its polarization can be determined without lead clarification, in comparison with the same filtrate after lead clarification. Portions of the filtrate, corresponding to 50 ml. volume, were weighed out. One of these portions was diluted to 100 ml., giving a normal solution, and this was read without further treatment. A second portion was first clarified with lead subacetate solution, made to the 100-ml. mark, filtered, and read; a third portion was first diluted to the mark, clarified with dry lead subacetate, filtered, and read. The results of these tests are shown in the following table, in which columns 3 and 4 give the increase in polarization over that obtained without any clarifying agent whatever.

1 Origin of Samples	2 No. of Samples	3 Pol. Inc. Pb Soln.	4 Pol. Inc. Dry Pb	5 Pb Soln. −Dry Pb
Cuba	26	0.133	0.098	0.035
Puerto Rico	17	0.123	0.050	0.073
Philippines	20	0.080	0.053	0.027
Hawaii	22	0.079	0.002	0.077
Louisiana and Florida	6	0.117	0.067	0.050
	91	0.105	0.054	0.051

The volume error (column 5) averages only 0.051, for reasons explained above. But it is also found that, even when dry lead subacetate is used for clarification, there is still a residual plus error, amounting to about 0.05° V., on the average.

The volume error due to clarification with lead subacetate solution, and shown to be about 0.1° V. by the first series of tests, is of the same

[77] *Arch. Suikerind.*, 41, I, 425 (1933); also 42, 116 (1934).
[78] *Z. Zuckerind. čechoslovak. Rep.*, 58, 49 (1933/34).

magnitude, but in the opposite direction, as the error in the Herzfeld-Schönrock saccharimeter scale. These errors thus counterbalance each other, and the reading of a raw sugar on the Herzfeld-Schönrock scale after clarification with lead solution represents on the average the true polarization within the limit of error of such readings, if the residual error just mentioned is disregarded. But if the Bates-Jackson scale is used, with clarification by lead solution, the polarization found is on the average 0.1° too high. This can be corrected again by using dry lead subacetate for clarification, which brings the reading back to the correct figure. This explains the ruling of the International Commission, given on p. 304, for the method of clarification to be used with either of the two saccharimetric scales.

Browne[79] has investigated the effect of clarification with lead subacetate solution, and with dry lead subacetate, upon the polarization of sorgo sirup, maple sirup, apple sirup, and a blend of commercial glucose with cane sirup. In all these sirups the lead subacetate solution gave a large increase in polarization, averaging 0.232, over polarization without clarification. The increase with dry lead subacetate averaged 0.147, showing that the volume error alone amounted to 0.085, similar to the figure found for raw sugars. The remaining error of 0.147 is probably due to precipitation of fructose and to increase in the dextrorotation of some of the optically active constituents of the solution.

The selection of an appropriate clarifying agent is one of the most important operations of saccharimetry, and in making his selection the chemist must be governed by the requirements of each particular case. Rapid filtration and brightness of clarification are factors which must be considered as well as minimum degree of error. Beginning with products of highest purity alumina cream alone should be used wherever possible. With products of slight discoloration, when alumina cream is insufficient, neutral lead acetate solution should be tried. When alumina cream and neutral lead solution fail, lead subacetate or basic lead nitrate or neutral lead acetate with hypochlorite may be employed; dry lead subacetate will usually give more accurate results with sugar cane and other products containing fructose. Animal charcoal or hydrosulfites should be used only as a last resort, when other means of clarification have failed. The smallest possible quantity of clarifying agent should always be used.[80]

[79] *J. Assoc. Official Agr. Chem.*, **18**, 157 (1935).

[80] The literature on clarification is completely reviewed in "Methods of Decolorizing Sugar Solutions for Analysis," a monograph by Nakhmanovich and Berman (Russian, with English summary). Summary reprinted in *Facts About Sugar*, **24**, 1142 (1929).

POLARIZATION OF SUGAR PRODUCTS CONTAINING INSOLUBLE MATTER

In the analysis of juices, sirups, massecuites, molasses, and sugars the chemist has to deal with substances which usually contain little or no insoluble matter.[81] The work of polarization becomes more complicated when considerable insoluble material is present, as happens in the analysis of fruits, tubers, stalks, and other vegetable substances, or in the examination of filter-press cake, scums, and other sugar-house residues.

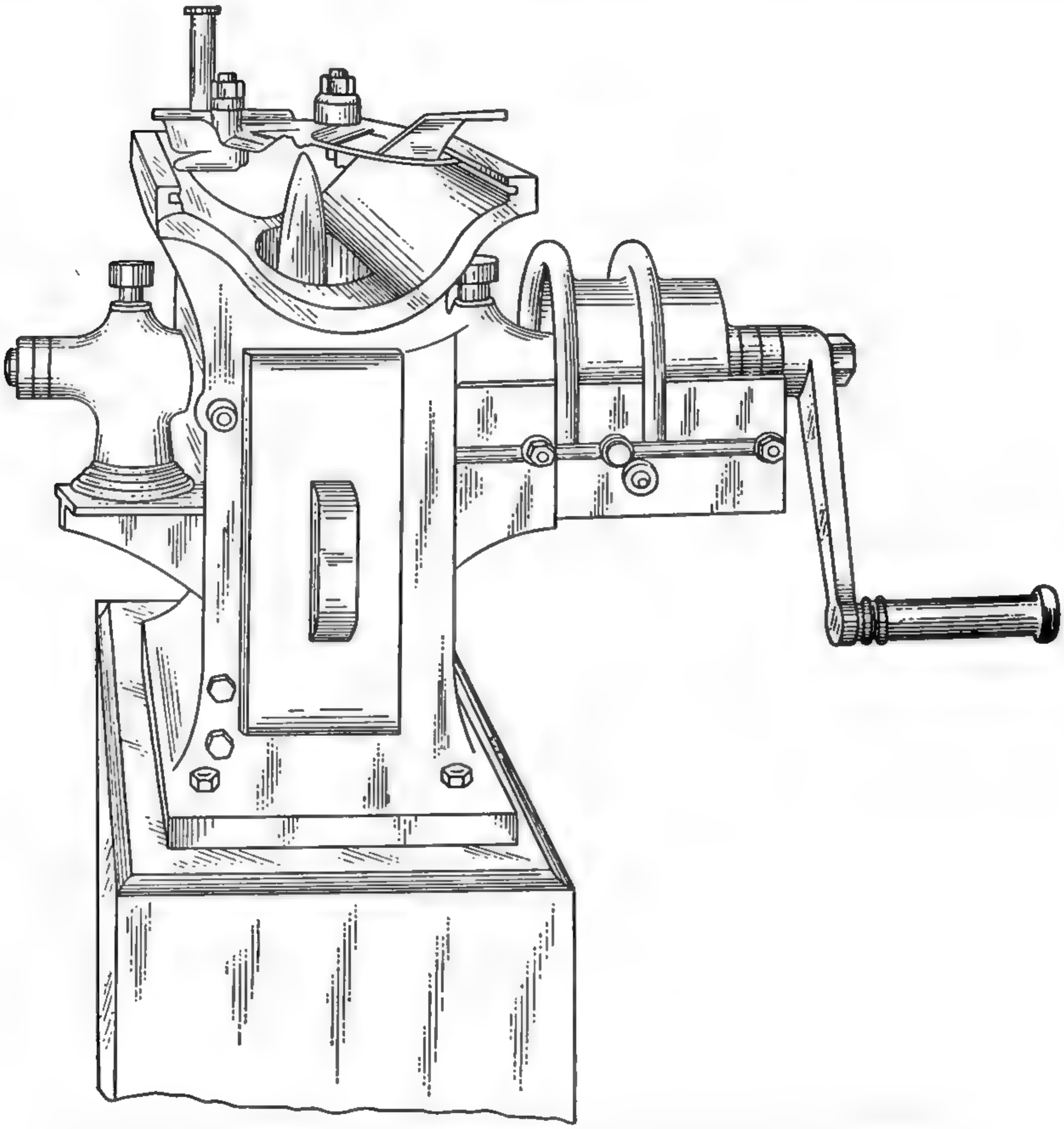
The methods of polarization of succulent plant materials may be divided into three general classes, namely, methods: (1) of expression; (2) of extraction; and (3) of digestion. As an illustration of these several methods the polarization of sugar beets offers a good and classical example.

Preparation of Samples for Analysis. Sugar beets, sugar cane, fruits, etc., must first be reduced to a finely divided condition. For this purpose any of the numerous rasps, shredders, graters, etc., may be employed, provided that the cellular tissue is thoroughly disintegrated and that no losses occur through leakage of juice or evaporation. A few of the machines that have been found practical and efficient will be described.

The Keil-Dolle Segment Rasp. This apparatus, Fig. 174, is the standard machine used in beet-sugar factories in the United States and is also widely employed in Europe. It was designed by Kiehle, and it is known under various names, in Germany as the "Hering" rasp. It may be run by hand but is usually power-driven. The essential part is the conical rasp which revolves at 1200 to 1400 revolutions per minute. The teeth must be sharp, and the points must present an even surface to deliver pulp of the necessary fineness. The beet is held firmly against the rasp in such a position that a wedge-shaped segment is removed, the edge of which coincides with the axis of the beet. But the pressure used must not be such as to retard the rotation of the rasp. The segment from the first beet is taken at the smaller diameter, from the second beet at the larger diameter, and so on alternately, to obtain a representative sample. Small and broken beets should be rasped in proportion to their number in the sample. The

[81] In experiments upon 60 samples of raw sugar Hardin (*Ind. Eng. Chem.*, 16, 55 [1924]) found from 0.017 to 0.423, averaging 0.159, per cent of water-insoluble matter. This causes an average error in polarization of +0.021 sugar degree, ranging from +0.001 to +0.057°. This error, though small, is of some significance in commercial transactions, where buyers and sellers employ averages to the fourth decimal in settlement of their transactions.

pulp is collected in a pan underneath the rasp. After the sample has been ground the pulp adhering to the rasp is removed into the pan with a fiber brush. The contents of the pan are transferred to a round-bottom enameled bowl by means of a reinforced rubber spatula, and



(Reproduced with permission from Frühling-Spengler, "Anleitung zu Untersuchungen," p. 176.)

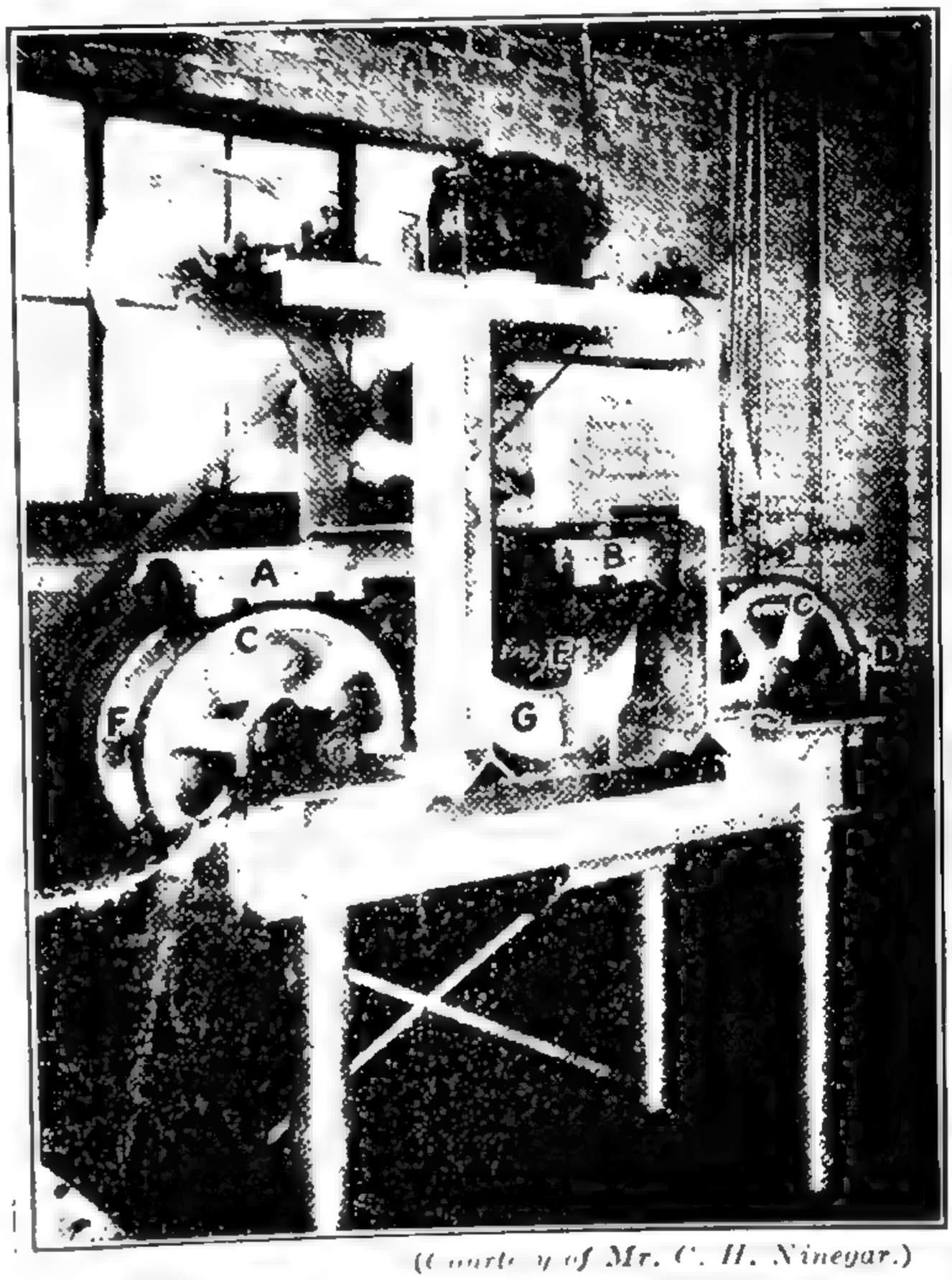
FIG. 174. Keil-Dolle segment rasp.

the sample is thoroughly mixed in the bowl with an open wire beater. All these operations must be carried out as rapidly as possible to avoid evaporation of the sample.

The rasp should be washed and scrubbed frequently with hot water and then dried. From time to time it is cleaned with dilute acid (1

part concentrated hydrochloric acid and 1 part water) to remove a deposit of calcium oxalate which gradually forms on the surface.

The quality of the pulp is checked by comparative digestions with the hot- and cold-water digestion process in order to see that the pulp is fine enough for cold-water digestion.



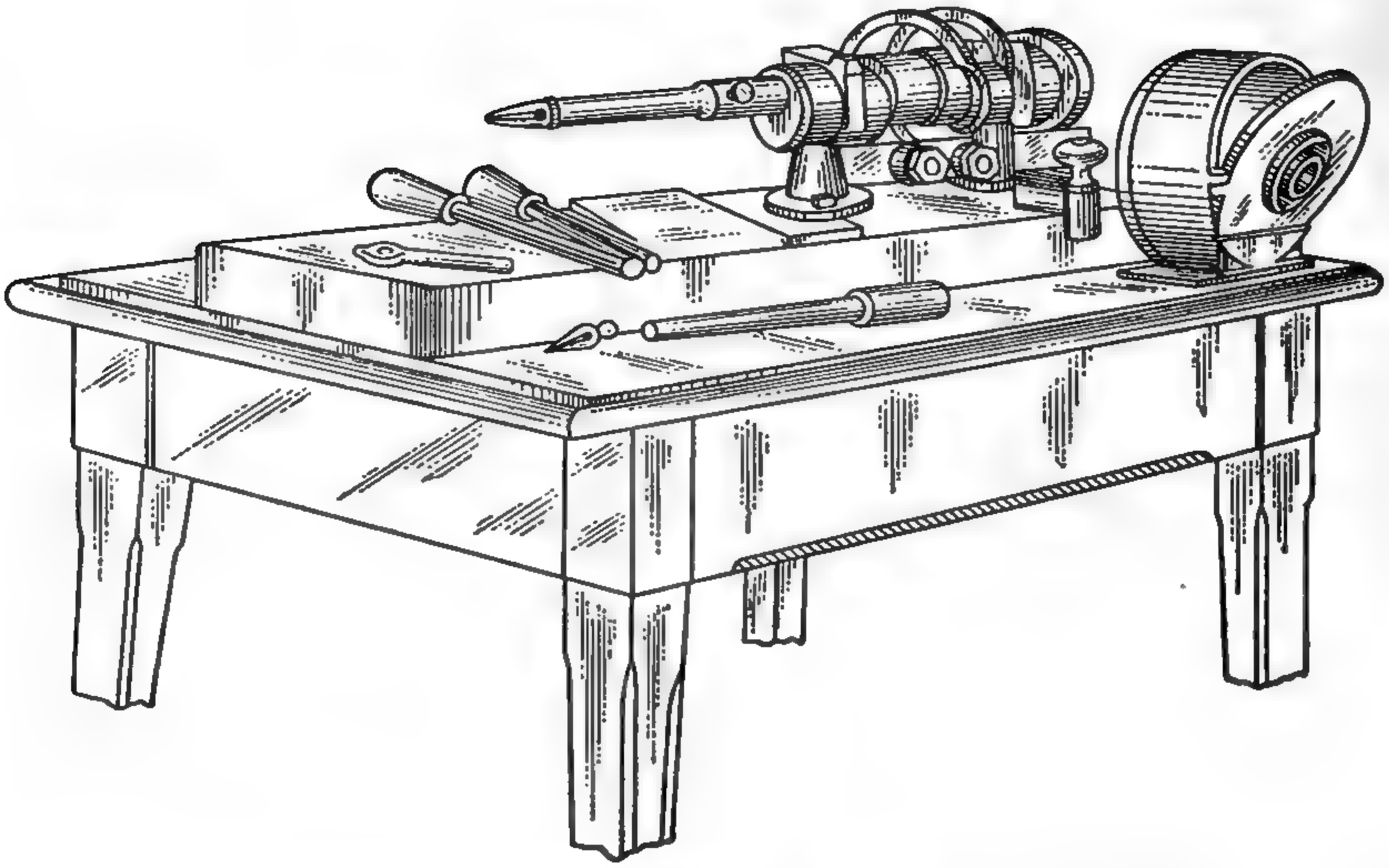
(Courtesy of Mr. C. H. Ninegar.)

Fig. 175. Ninegar rasp.

The Ninegar Rasp.[82] In this improved design, Fig. 175, the rasp disk of the Keil-Dolle apparatus is retained, but the beets are conveyed mechanically to and over the rasp, thereby speeding up and standardizing the operation and reducing hand labor. The machine is equipped with five beet holders (*A*, *B*, etc.), consisting of slotted cones which are welded into rigid frames spaced equally on a sprocket and chain conveyor (*C*). The beets are dropped into the cones of the beet holders as they come up around the front sprockets (at *D*) and are held securely centered with respect to the edge of the Keil disk (*E*). As they pass over the disk at a uniform speed of 135 feet per minute a segment is removed from each beet from the surface to the center through the entire length of the beet in the form of a very fine pulp. As the beet holders pass over the rear sprockets the sampled beets are engaged by the ejector wheel (*F*), revolving approximately three times as fast as the sprockets, which throws the beets out of the beet holders into a flume, elevator, or suitable disposal mechanism. The sample is collected in a receiving pan (*G*).

The Keil disk is driven by two V belts directly from the 3-horsepower motor. A clutch is provided through which the conveyor mechanism is driven and when disengaged permits moving the beet-holder conveyor

[82] Private communication from Mr. Ninegar.



(Reproduced with permission from Frühling-Spengler, " Anleitung zu Untersuchungen," p. 177.)

FIG. 176. Keil-Dolle boring machine.

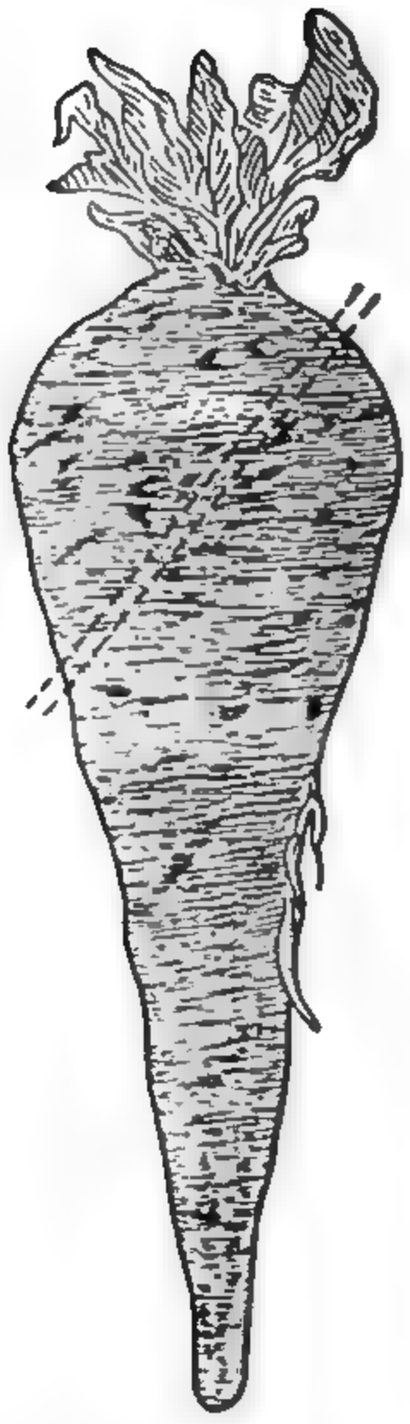


FIG. 177. Showing direction of boring in sampling sugar beets.

by hand for cleaning the machine. The capacity of the machine with one man feeding beets into the holders and one man mixing samples is 60 to 90 samples per hour, each sample consisting of 20 to 25 beets. Excellent checks are secured on samples by the hot and cold digestion methods.

Other rasps frequently used in Europe are those of Perner and of Staněk.

The Keil-Dolle Boring Machine. This machine, Fig. 176, differs from those described above in that it drills a hole through the beet in an oblique direction, as shown in Fig. 177, in order to secure a fair sample with least injury to the beet. The essential feature of this apparatus is a hollow detachable bit, the construction of which is illustrated in Fig. 178. The conical rasp at the end, revolving at a speed of 3000 revolutions per minute, reduces the substance of the beet to an extreme degree of fineness and at the same time forces the pulp through a small opening into the cavity within. When only single beets are examined (as in the selection of " mother beets " for seed selection) the bit is detached after each boring and a new one screwed on. The bits

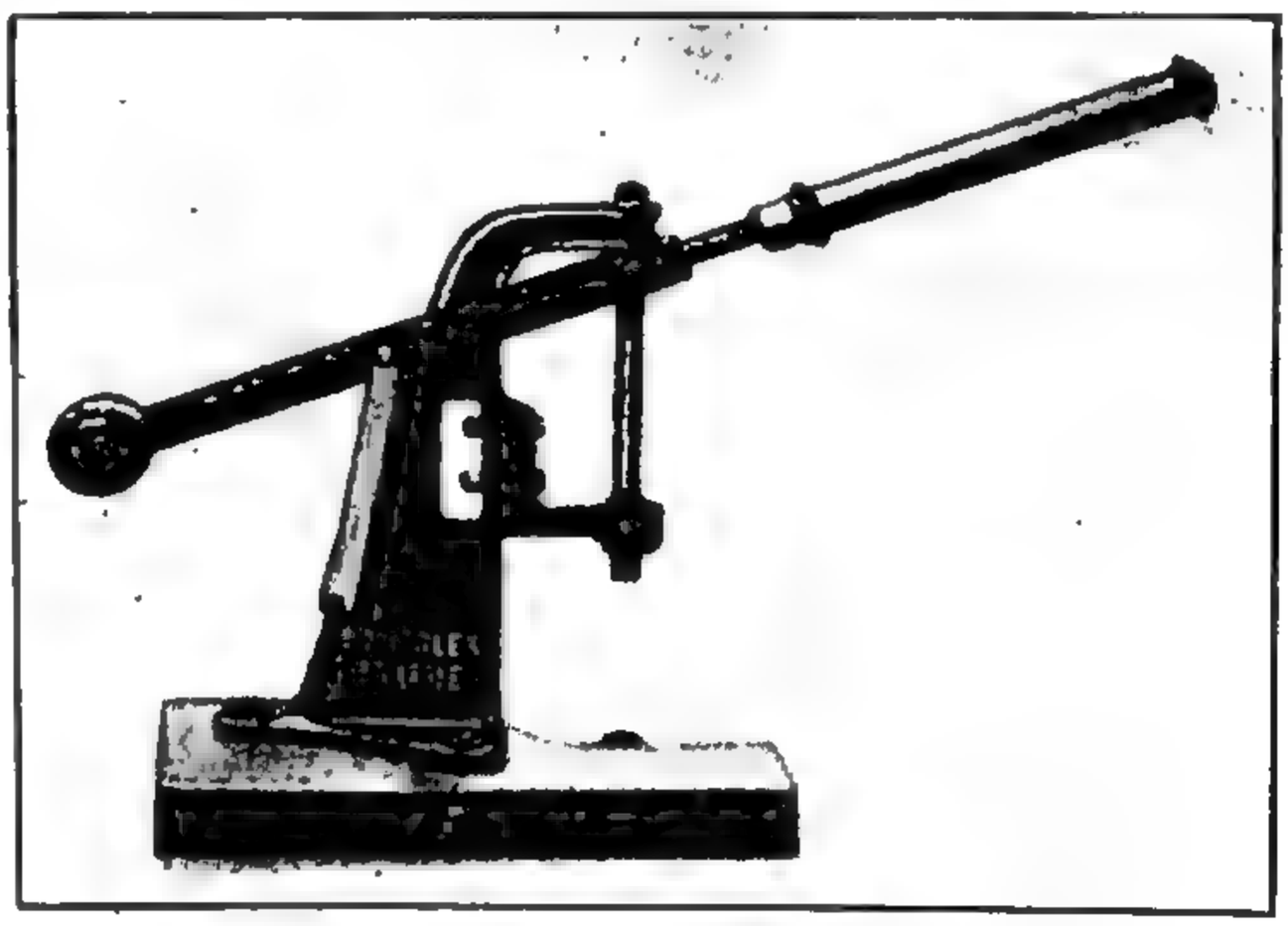
are numbered, and to collect the sample the conical rasp is removed and the pulp (from 8 to 14 g., according to the size of beet and length of boring) forced out with a rod. In sampling large numbers of beets the bit is kept in constant use, the pulp being discharged in a continuous stream into a covered container at the end of the apparatus.

(Reproduced with permission from Frühling-Spengler, "Anleitung zu Untersuchungen," p. 177.)

FIG. 178. Showing details of bit for the Keil-Dolle boring machine.

Simpler and cheaper boring machines which cut a solid core out of the beet are also frequently employed. They are constructed on the principle of the familiar cork borer and are actuated by a lever. Such a machine, designed by Herles, is shown in Fig. 179. The beet to be bored is held obliquely, as illustrated in Fig. 177. The solid core samples must be further disintegrated to prepare them for analysis.

The boring machines do not furnish a true sample of the entire beet, but they are useful for comparative tests in beet-breeding work.



(Courtesy of Dr. F. Herles.)

FIG. 179. Herles's beet corer.

If mechanical rasps are not available, longitudinal sections may be cut from each beet and ground with a hand grater, but a coarse pulp is thus obtained.

Fresh cossettes from the beet-slicing machines must also be ground to prepare them for analysis. This is usually done with a meat chopper. The "Enterprise" chopper No. 41 or some similar type is recommended in the United States. It runs at a speed of 300 revolutions per minute and is provided with a plate having $\frac{1}{8}$-inch perforations. In Czechoslovakia the "Keystone" machine, with ten holes in the plate, is favored.

Samples that have been prepared by the mechanical rasps mentioned above are in a sufficiently fine division to give up the sugar readily to alcohol or water even at room temperature. But solid beet cores, or samples ground with the meat chopper or by hand grating, must be converted into finer pulp if the cold-water methods are to be used for the analysis. The following mechanical aids may be employed for grinding to a fine pulp:

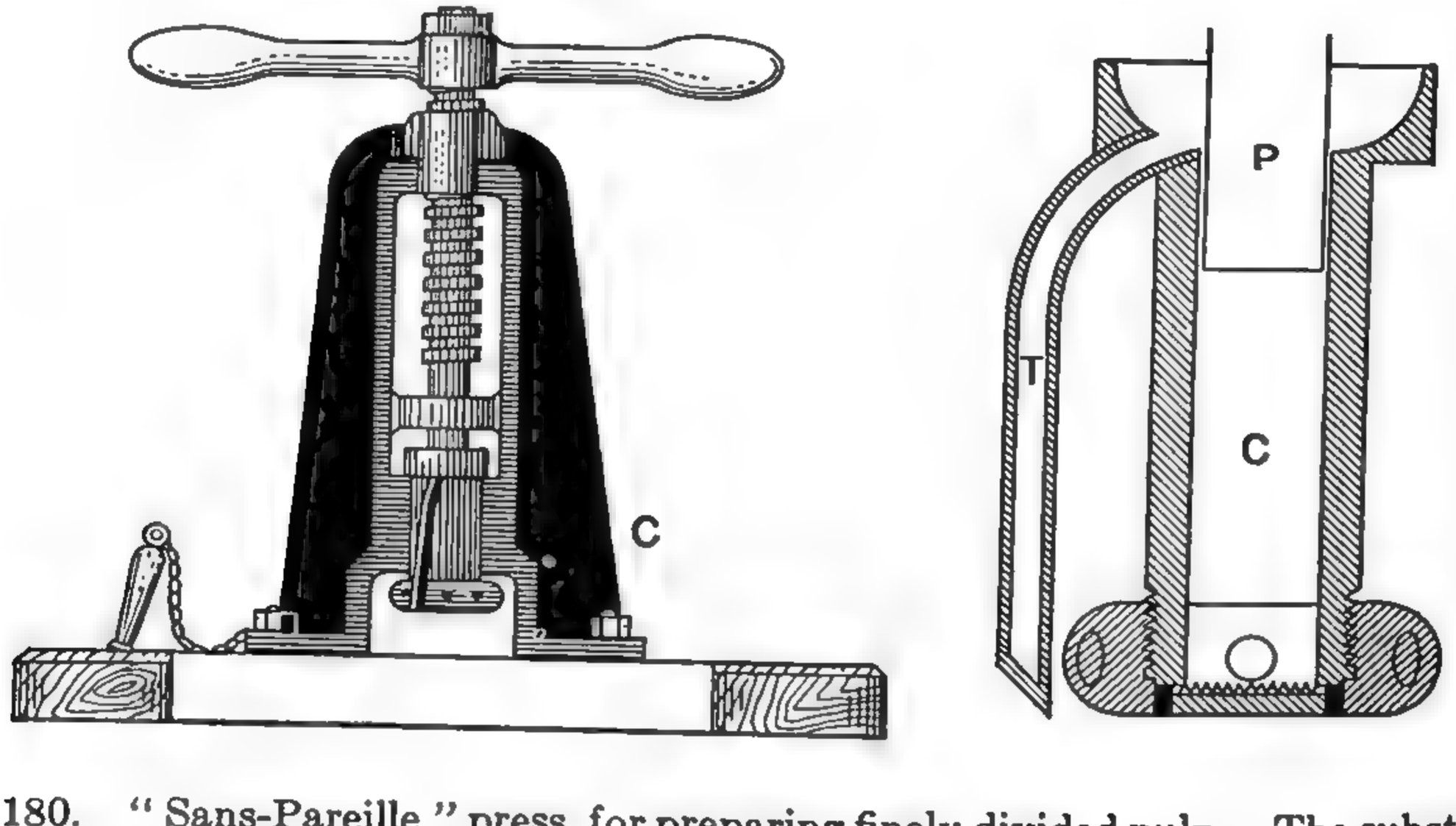


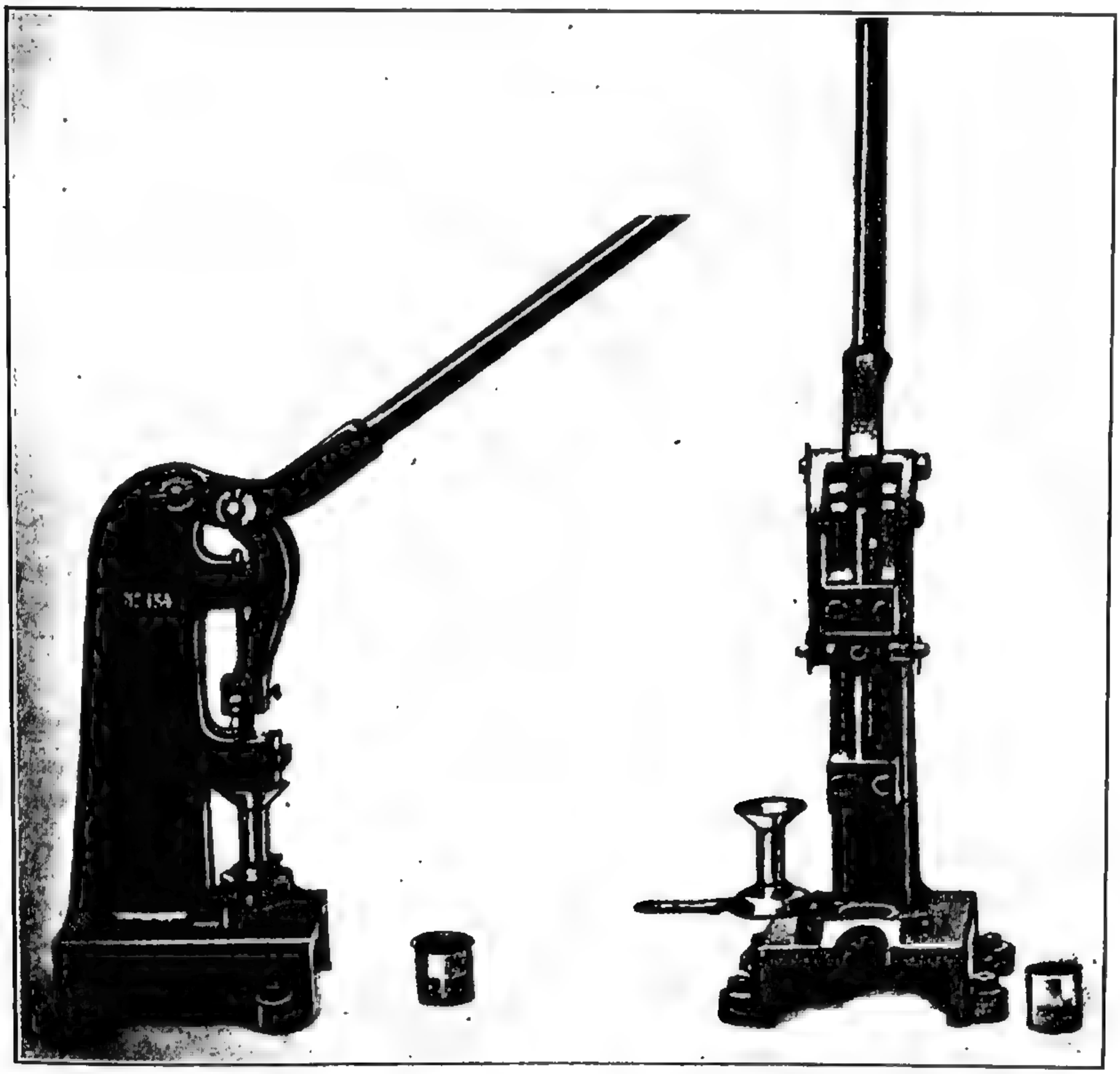
FIG. 180. "Sans-Pareille" press, for preparing finely divided pulp. The substance which is placed in the cell *C*, is forced in a semiliquid condition by the piston *P* through the fine openings at the bottom into a container underneath; the latter also receives any overflow of juice which escapes by the outlet *T*.

The "Sans-Pareille" Press. This press, Fig. 180, has a hollow steel cylinder *C* in which the sample to be ground is placed. As the piston *P* is moved down the sample is pressed against a number of fine sharp teeth at the lower surface of *C*, and the disintegrated pulp passes through the little channels between the teeth and then through five narrow openings in the bottom, and is collected in a container underneath. The container also receives the overflow of juice which escapes by the outlet *T*.

The Herles Press.[83] In this apparatus, Fig. 181, the beet sample is disintegrated by being pressed through sieves. The cylinder which receives the sample can be unscrewed from its base. A perforated steel plate is inserted, and on this is placed a fine brass sieve. Both sieves have cone-shaped holes, and the surface with the narrow openings must be turned upward. The cylinder is screwed tight into the

[83] Published with the permission of the inventor, Dr. Franz Herles.

base so that the pulp cannot escape around the edges of the sieves. The sample is placed in the cylinder, and if it consists of cossettes or pulp it is tamped down with a wooden rod. The cylinder is now swung from the position shown in Fig. 181, *b*, to that in *a*, fastened

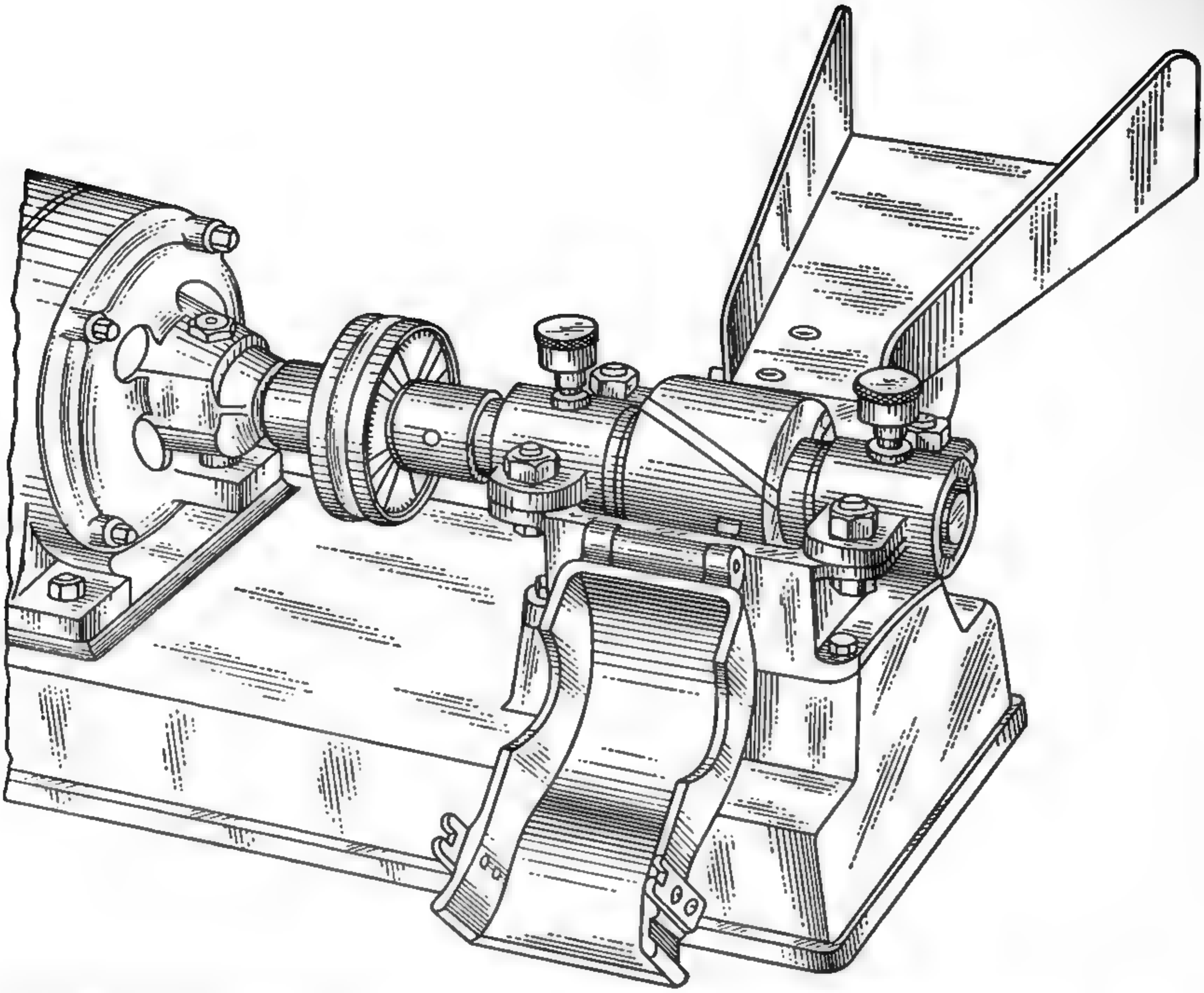


(*Courtesy of Dr. F. Herles.*)

a *b*

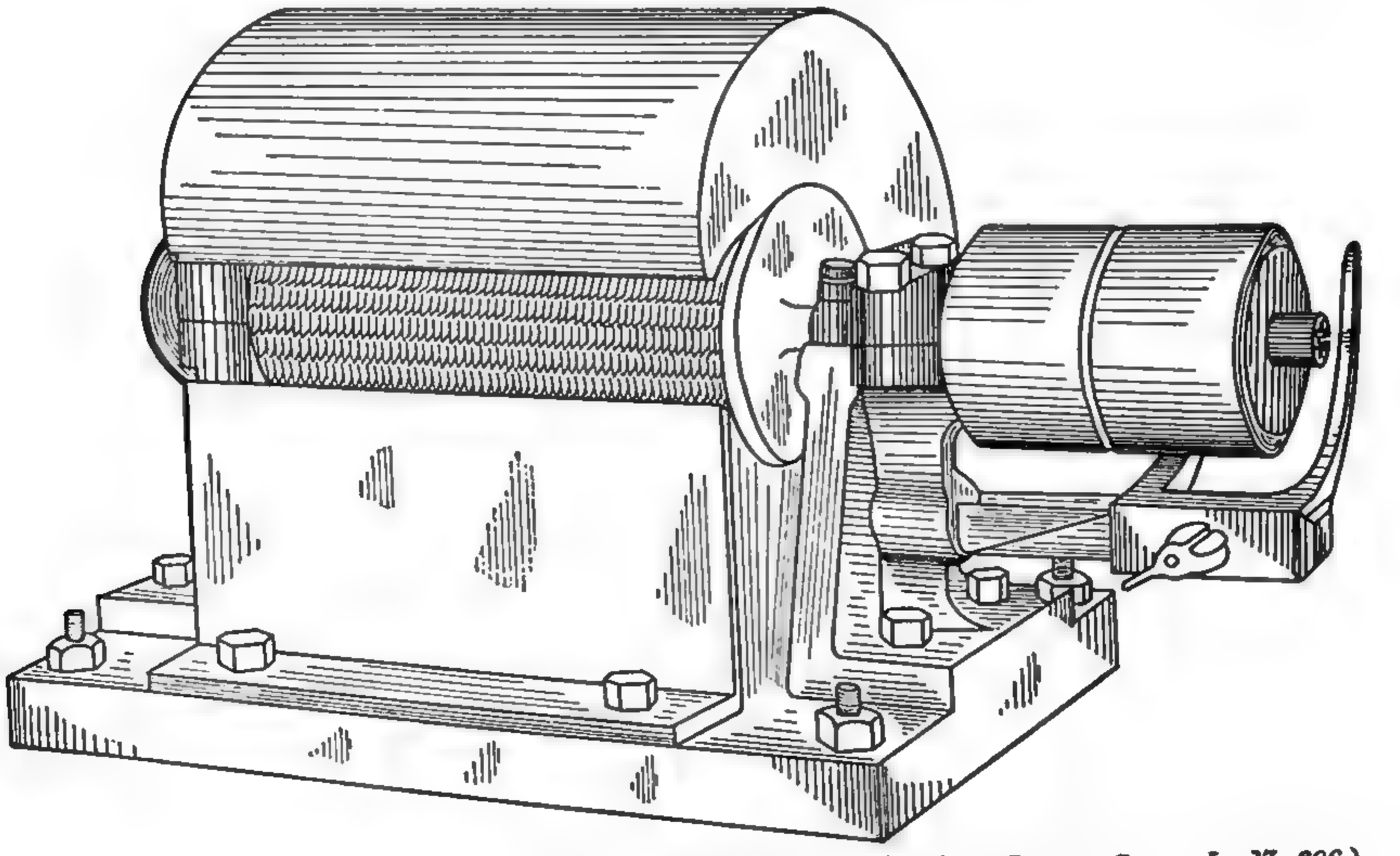
FIG. 181. Herles's press for pulping beet samples: (*a*), side view, with lever pressed down; (*b*) front view, with lever raised.

under the piston, and the piston is pressed down. The fine pulp is collected in a cup underneath. Large samples are passed through the press in successive portions, and this can be done very rapidly. Difficulty in pressing down the piston is usually caused by woody beets. In such cases the sieves must be taken out at once and thor-



(*Reproduced with permission from Spencer-Meade, " Handbook for Cane-Sugar Manufacturers," p. 307.*)

FIG. 182. Warmoth-Hyatt cane shredder.



(*Reproduced with permission from Intern. Sugar J., 37, 306.*)

FIG. 183. " Cutex " cane fibrator.

oughly cleaned to remove the woody fibers, and the operation is repeated with a smaller sample. Seed beets that are found to be woody are discarded entirely because this characteristic is undesirable and usually hereditary.

Both the Sans-Pareille and Herles presses handle only small samples. Rossée and von Morgenstern[84] have designed a press by which 500-g. samples of cossettes can be ground, without previous treatment, to the finest pulp in 1 minute.

Preparation of Sugar Cane for Analysis. Sugar cane requires very powerful machines for disintegration, because of its hard rind. An efficient machine for this purpose is the Warmoth-Hyatt shredder, Fig. 182. The cutting mechanism consists of a number of knives fastened to a rapidly rotating shaft. The hinged cover is closed and bolted down, and the cane, cut into short lengths, is fed into the hopper. The shredded cane is collected in a pan placed underneath.

Another form of " fibrator " is the " Cutex " disintegrator,[85] Fig. 183. It has 46 saw-disks, placed close together and revolving at 1500 revolutions per minute or more. The cane is introduced through the opening in front, pressed against the saws, and moved across the cutting edge. The shredded cane falls into a box below. This machine or similar ones are widely used in Australia, India, and other countries.

Meat-slicing machines, such as Enterprise No. 23, are employed in some places with good results.[86] In others the cane is cut into small pieces which are then chopped in an Athol meat-chopping machine. If no machines are available the cane may be sliced with a wood plane turned upward.

I. Determination of Sugar in Plant Products by Expression of Juice

The determination of the sugar in sugar beets by polarization of the expressed juice was formerly quite common but has now given place to more accurate methods of analysis.

Assuming (as is incorrect) that the sugar, amides, albuminoids, salts, gums, and other water-soluble solids of the beet are in the same condition of solution within the beet as in the expressed juice, and letting M = the per cent of water-insoluble matter or " marc " and $100 - M$ = the per cent of juice, then the sugar content (S) of the beet can be

[84] *Deut. Zuckerind.*, 53, 565 (1928); 54, 990 (1929); *Centr. Zuckerind.*, 38, 1138 (1930).

[85] *Intern. Sugar J.*, 37, 306 (1935).

[86] See also Kerr, *Proceedings 9th Annual Conference, Queensland Society of Sugar Cane Technologists* (1938), p. 111.

calculated from the polarization (P) of the expressed juice by the formula

$$S = \frac{P(100 - M)}{100}$$

Example. The expressed juice of a sugar beet gave a polarization of 16.2° V. for the normal weight: the beet contained 4.6 per cent of marc. Required the per cent of sugar in the beet.

$$S = \frac{16.2\ (100 - 4.6)}{100} = 15.45 \text{ per cent}$$

The above method is, of course, equally applicable to the analysis of sugar cane, fruits, and other succulent plant substances.

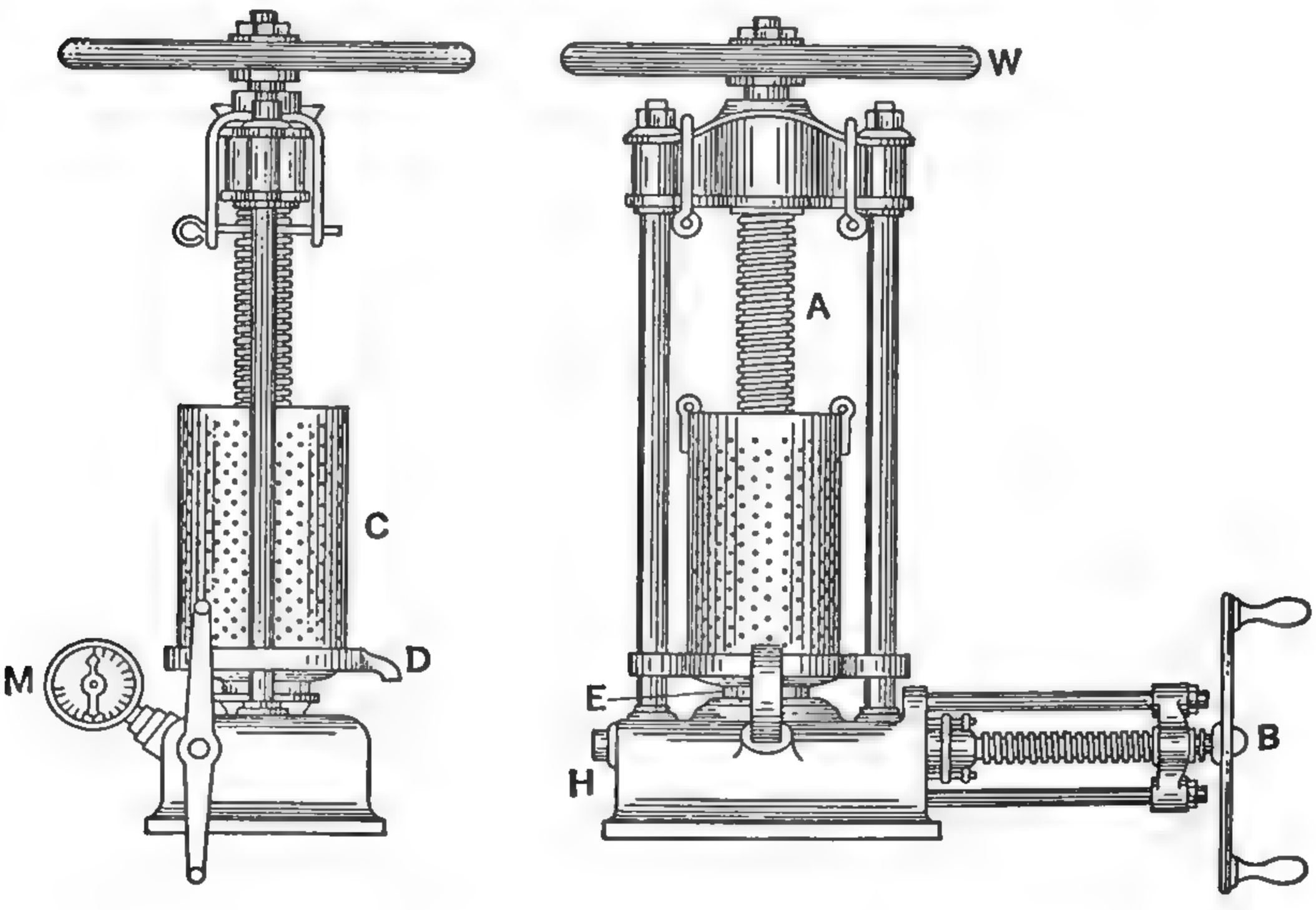


FIG. 184. Laboratory hydraulic press for expressing juices.

Method of Expressing Juice. For expressing the juice from the pulp of sugar beets, sugar cane, etc., any suitable form of hand press may be used. The small hydraulic press shown in Fig. 184 is one of great efficiency and is a piece of apparatus almost indispensable in a sugar laboratory.

The pulp to be pressed is placed in a strong sack inside the perforated container C, and covered evenly with a heavy metal disk. By

the turning of the wheel *W* the screw *A* is driven downward as far as possible upon the disk, thus squeezing out through the openings of *C* a considerable part of the juice, which escapes by the spout *D* into a can or other receptacle. The horizontal hydraulic screw *B* is then turned inwards. This screw, operating by means of glycerol which fills the hollow base *H*, forces the piston *E* upwards and removes by vertical pressure a second fraction of juice. The final pressure, indicated by the manometer *M*, can be raised to 300 atmospheres. The juice, as the pressure increases, is of gradually diminishing purity; it is important, therefore, that all the runnings should be well mixed before the sample for polarization is taken.

A determination of the insoluble cellular matter, or marc, is necessary before the percentage of sugar in plant substances can be calculated from the polarization of the expressed juice. For rough purposes of estimation a constant percentage of 5 per cent or 4.75 per cent marc is sometimes assumed for the sugar beet and 10 per cent or 12 per cent fiber for the sugar cane. Such figures, however, have no exact value, as the percentage of cellular matter varies considerably according to the age of the plant, dryness of the season, and many other conditions. For grinding cane stalks a small cane mill is useful.

Determination of the Dry Marc. The general definition of marc as the water-insoluble portion of the beet is inadequate because the quantity of dissolved material varies with the temperature of the water used, with the length and manner of treatment, with the particle size of the beet pulp, and with the ratio of water to pulp. Treatment with cold water is slow because it is dependent on diffusion through the cell walls unless they have been broken. A temperature of about 65° C. is required to destroy the protoplasm and to facilitate the extraction of the soluble substances. But at this temperature the hemicelluloses and the pectin are attacked and converted into water-soluble substances. If alcohol is used for the extraction, as has been advocated by some investigators, the albumen is coagulated, and a part of the salts is not extracted. The marc obtained by alcoholic extraction is therefore usually higher than that found by water extraction.

The method generally used for the determination of the dry marc is based on a procedure originally suggested by Stammer.[87] Twenty grams of the finely divided beet sample is digested in a beaker for half an hour with 300 to 400 ml. of cold water. The solution is removed by suction through an inverted filter immersed in the liquid. The filter, Fig. 185, consists of a cylindrical stopper of piano felt inserted in the widened end of a glass tube. The digestions and filtra-

[87] *Z. Ver. deut. Zucker-Ind.*, 32, 634 (1882).

tions are repeated until the filtrate is free from dissolved substances, as disclosed by color and taste, or by the α-naphthol reaction. The residue is treated with boiling-hot distilled water and collected on a weighed, dried filter paper. It is washed two or three times with 90 per cent alcohol and finally with a little ether. After evaporation of the ether in the air, the filter paper and marc are dried first at a low temperature and then at 100 to 110° C. to constant weight, being weighed each time in a stoppered weighing bottle. The filter paper and marc are ashed; the ash is corrected for that of the filter paper, and the ash thus found, representing sand and other extraneous matter, is deducted from the weight of the dry marc. The result, multiplied by 5, gives the percentage of dry marc in the beet.

(*Reproduced with permission from Frühling-Spengler, "Anleitung zu Untersuchungen," p. 192.*)

FIG. 185. Filter tube used for determining dry marc.

Claassen[88] has expressed the opinion that a definition of marc can be established only by agreement among sugar technologists and plant physiologists, but that practical considerations should be given full weight. He defines marc as the residue remaining after complete extraction of the sugar and of the easily soluble nonsugars under conditions similar to those in factory operation but in as short a time as possible to prevent the formation of soluble substances by the decomposition of hemicelluloses, pectin, etc. The method of determination proposed by Claassen is as follows:

Twenty-five grams of the ground beet sample is transferred to a beaker provided with a mark at the 400-ml. level. Boiling water is poured over the sample to the mark. The pulp is digested for 2 minutes and then rapidly separated in a Büchner funnel. The pulp is put back in the beaker and the operation is repeated three times. After the fourth digestion the marc is collected on a weighed filter paper, washed with a little alcohol, and dried for 6 to 8 hours at 105 to 110° C.

A method in which the dry marc, polarization, refractometer solids, and ash are determined upon one and the same sample has been described by Staněk and Pavlas.[89]

Fiber Determination in Sugar Cane. Cold water is generally used for this determination. The method of the Association of Hawaiian Sugar Technologists is given as an example:[90]

[88] *Z. Ver. deut. Zucker-Ind.*, 66, 359 (1916).
[89] *Z. Zuckerind. čechoslovak. Rep.*, 62, 357, 365 (1937/38).
[90] "Methods of Chemical Control," 2nd ed., p. 33, 1931.

Transfer the weighed, finely divided sample, representing about 200 g. of cane, to a strong linen bag and tie with heavy thread. (Twine forms knots which are likely to rupture the bag.) Wash in running water until the washings are clear, squeeze out surplus water, place the bag in a heavy canvas bag and press in a screw press (p. 346). From 600 to 1000 pounds per square inch is a suitable pressure. The pressed mass is then broken up, the bag examined to see that no holes have been made during pressing, treated with cold water for at least 2 minutes, and repressed. Five or six alternate soakings and pressings will extract a properly prepared sample. The thoroughness of the extraction may be roughly checked by crushing some of the larger particles between the teeth. If juice cannot be detected by taste in the larger pieces the extraction may be considered complete. Dry sufficiently that the fiber may be easily removed from the bag, transfer to a tray, removing the particles adhering to the bag by rubbing, and dry at 125° C. Three hours' drying should suffice. Weigh quickly to avoid absorption of moisture. Instead of pressing, the sample can be extracted by washing in cold running water for 24 hours.

In the official method prescribed in Queensland[91] a 100-g. sample of fibrated cane is extracted, in a calico bag, for 1 hour with cold running water, and for another hour with circulating boiling water, the bag being squeezed at intervals during the treatment. The bag is then pressed to remove most of the water, and dried to constant weight at 100° to 105° C. The fiber is removed from the bag, the bag reweighed, and the weight of the fiber found by difference.

A method described by Spencer[92] employs a metal extractor with a siphon, similar to a Soxhlet extractor. The shredded cane is treated repeatedly with cold and finally with hot water until all the soluble matter has been extracted by percolation. After the surplus water is drained off, the fiber is dried and weighed.

Errors of Expression Method. Several sources of error are involved in the determination of sugar in plant substances by analysis of the expressed juice. In the first place a considerable amount of juice, varying from 10 per cent to 30 per cent, according to the efficiency of the press, is not eliminated, and this residual juice, containing a larger amount of albuminoids, pectin, etc., is of much lower purity than the part first expressed. This excess of impurities in the unexpressed juice is washed out, however, in the marc determination. The polarization of the expressed juice is thus higher than that of the composite juice of the entire plant. (See under Distribution of Water, p. 351.)

[91] "Laboratory Manual for Queensland Sugar Mills," 2nd ed., p. 77, 1939.

[92] "Spencer's Handbook for Cane-Sugar Manufacturers," 7th ed. by Meade, p. 309, 1929.

The second source of error already mentioned on p. 347 is the extraction during the marc determination — by excessive amounts of cold water, but more especially by hot water, alcohol, and ether — of variable amounts of hemicelluloses, wax, oil, and other substances which are, strictly speaking, not juice constituents and should therefore be included in the marc. The percentage of juice is thus estimated too high, and a plus error is introduced in the calculation. Except for the disadvantage of loss of time in drying, the use of alcohol and ether as dehydrating agents should be omitted in the marc determination, and cold water alone be used for extracting.

"Colloid" or "Imbibition" Water. A third source of error to be mentioned is the much-debated question of "colloid" or "imbibition" water, by which is meant water, in a more or less hydrated form, in combination with hemicelluloses and other plant constituents. This imbibed water contains no sugar in solution, and, it being expelled from the pulp upon drying, the percentage of sugar-containing juice is overestimated.

Heintz,[93] in 1874, showed that, when the air-dried and sugar-free marc of beets was placed in sugar solutions, water was imbibed, thus leaving the sugar more concentrated and increasing the polarization. In the following experiments by Heintz, air-dried beet marc which had been washed completely free from sucrose was treated 16 hours in a cool place with solutions containing a normal and half-normal weight of sucrose, in the proportion of 1 g. marc to 20 ml. of solution.

	Half-Normal Weight	Normal Weight
Polarization before marc treatment.....	49.8	99.6
Polarization after marc treatment.....	53.9	104.6

The observations of Heintz were verified in a different way by Scheibler,[94] who found that samples of sugar beets, the expressed juice of which polarized 14.5, had a marc content of 4.71 per cent. The percentage of sugar in the beets according to the formula

$$S = \frac{P(100 - M)}{100}$$

would be 13.82. Scheibler, however, found by his method of alcoholic extraction a percentage of only 13.1 or a difference of 0.72 per cent.

[93] *Z. analyt. Chem.*, **1874**, 262.
[94] *Z. analyt. Chem.*, **1879**, 176, 256.

The percentage of sugar-containing juice in the beets, assuming that this juice is of the same polarization as the part expressed, is found by the formula, per cent juice $= 100\frac{p}{P} = 100\frac{13.1}{14.5} = 90.34$ per cent, in which p is the polarization of the beets by the extraction method and P the polarization of the expressed juice. The percentages of juice and marc being respectivly 90.34 and 4.71, there is left a remainder of 4.95 per cent, which Scheibler termed "colloid" water. This method of estimation, however, is based upon the assumption that the juice expressed is of the same composition as the combined juices of the beet, which is not exactly true.[95]

Distribution of Water in Plant Tissues. The distribution of the water in plant tissues has such an important bearing upon certain problems of sugar analysis that a short discussion of the question may be introduced with profit at this point.

Figure 186 shows a magnified cross section of a part of a sugar-cane stalk. The sugar-containing juice proper, represented by *S* (the vacuoles), constitutes the principal part of the cell contents in the thin-walled parenchyma or fundamental tissue and includes the greatest part of the water in the cane. Lining the walls and permeating through these cells are thin layers and threads of protoplasmic matter *P* which contains a considerable amount of water but is deficient in sugar. Running longitudinally through the stalk are large numbers of fibrovascular bundles whose ducts, *D*, are filled with water taken up from the soil. The water of these ducts may often be seen spurting from the end of a cane stalk as it passes between the rollers of a mill, and is found upon analysis to be almost free of sugar. Running parallel with the ducts are the sieve tubes *T* which carry in solution the

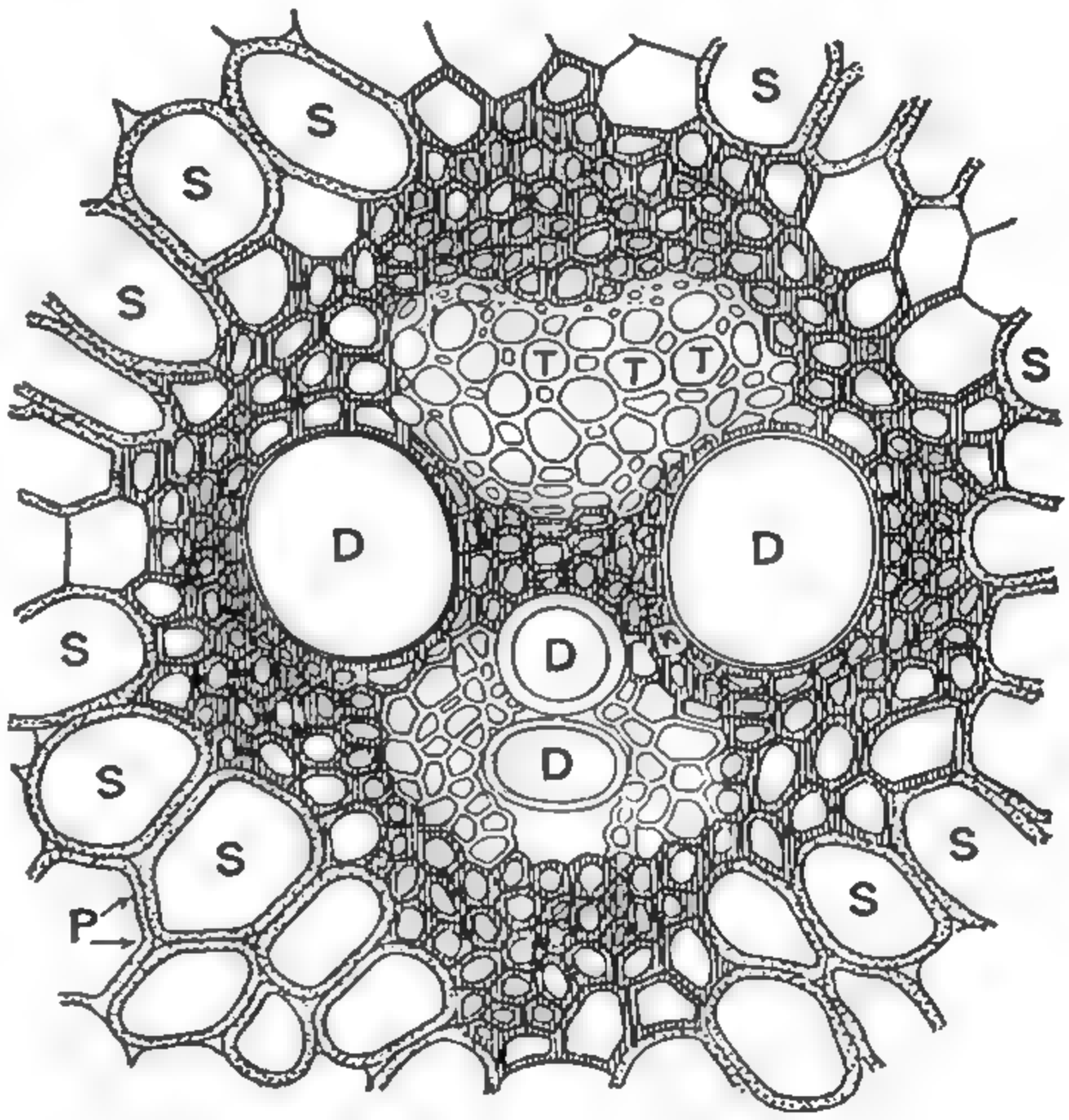


FIG. 186. Magnified cross section of sugar cane (protoplasmic lining *P* much intensified).

[95] For a very full discussion with bibliography of the subject of "colloid" water see Rümpler, "Die Nichtzuckerstoffe der Rüben," pp. 1–13, 1898.

products of assimilation from the leaf to the stalk. The water of these tubes contains reducing sugars but is deficient in sucrose. The cellular walls of the parenchyma and fibrovascular bundles consist of about 50 per cent cellulose, 20 per cent xylan, 5 per cent araban, 0.1 to 0.2 per cent galactan, and a remainder of lignin substances.[96] The pentosans named are partly present in the form of pectinogen, of which cane fiber contains about 1 per cent. The composition of beet marc, according to Smoleński,[97] is about 22 per cent cellulose, 73 per cent pectin, and 5 per cent albuminous substances. All these hold a certain amount of water in the imbibed or " colloid " form.

Variation in Composition of Juice from Different Mills. The pressings from the first rollers or crusher of a cane mill consist mostly of the sugar-containing juice *S* (Fig. 186). The pressings from succeeding rollers, where the pressure is greater, contain more and more of the protoplasmic juice *P* and the juice from ducts and tubes. The colloid water of the cellular substance is of course not affected by the milling.

The composition of the pressings from the different rollers of a cane mill is given in Table LIX.

TABLE LIX

COMPOSITION OF PRESSINGS FROM DIFFERENT ROLLERS OF A CANE MILL

	First Rollers	Second Rollers	Third Rollers
	per cent	per cent	per cent
Water	84.64	85.40	85.35
Sucrose	12.93	11.41	11.30
Reducing sugars	1.54	1.29	1.23
Ash	0.37	0.58	0.77
Albuminoids	0.18	0.50	0.58
Gums, acids, etc.	0.34	0.82	0.77
Total	100.00	100.00	100.00
Per cent extraction of cane	64.50	5.50	2.13

The pressed cane (bagasse) from the third rollers still contained over 60 per cent of water, corresponding to about 20 per cent of the total juice in the cane. If all this residual juice could be squeezed out by some inconceivable pressure, its sugar content would be much inferior to that of the pressings from the third rollers. It would, of course, be inaccurate to estimate the sugar content of the cane from the

[96] Browne, *La. Sugar Expt. Sta., Bull.* 91, p. 7, 1907; Farnell, *Intern. Sugar J.*, 25, 630 (1923).

[97] *Proc. 4th Intern. Congr. Agr. Industries*, 1935.

polarization of the first pressings; the same is also true, but to a much less degree, of the composite pressings of several mills.

The impossibility of obtaining by pressure a true composite sample of the different juices of a plant, the difficulty of estimating the true content of marc, and the uncertain influence of the colloid or imbibed water are the chief objections to the expression methods of sugar determination.

IIA. Determination of Sugar in Sugar Beets by Extraction with Alcohol

The method most accurate in principle for determining sugar in beets and other plant substances is extraction. In this process the sugar is washed out from the pulp and the extract made up to volume and polarized. The errors due to uneven composition of juices, faulty marc estimation, and colloid water are thus completely eliminated.

Scheibler's Alcohol-Extraction Method. The solvent most generally used for the extraction of sugar from beet pulp is 90 per cent ethyl alcohol. The method of Scheibler[98] as modified by Sickel,[99] and later again by Herzfeld,[100] is as follows:

A normal weight of finely prepared pulp is weighed rapidly in a weighing dish; a minimum amount of lead subacetate solution (3 ml. is usually sufficient) is added and thoroughly mixed with the pulp by means of a glass rod. The mixture is washed with 90 per cent alcohol into a flask of about 100-ml. capacity until the flask is about half full. A reflux condenser is attached, the flask placed in a water bath, and the alcohol gently boiled for 10 to 15 minutes. The contents of the flask are then transferred quantitatively with 90 per cent alcohol to the extraction cylinder *B* of a Soxhlet extractor, of which Fig. 187 shows six in the form of a battery. The bottom of the extraction cylinder is covered with a clean wad *D* of felt or cotton, or with a disk of fine metal gauze. The pulp is distributed evenly and loosely by means of a glass rod so that its upper surface is below the upper bend of the siphon tube *S*, and the glass rod is rinsed with 90 per cent alcohol. The top of the extraction vessel is then connected by means of a tight-fitting cork with the condensing tube *C*, and the bottom with the 100-ml. flask *F*. Instead of the Kohlrausch flask shown in Fig. 187, a flask of the form shown in Fig. 188 may be used to advantage because it allows more room for foam. The total quantity of alcohol must be chosen so that the flask is about three-quarters full.

[98] *Neue Zeitschrift*, 2, 1, 17, 287 (1879); 3, 242 (1879).
[99] *Neue Zeitschrift*, 2, 692 (1879).
[100] *Z. Ver. deut. Zucker-Ind.*, 59, 627 (1909).

The water in the bath, which should be fairly deep, is heated until the alcohol in the flask begins to boil vigorously. The vapor from the boiling alcohol passes upward through the side tube *A*, and condensing

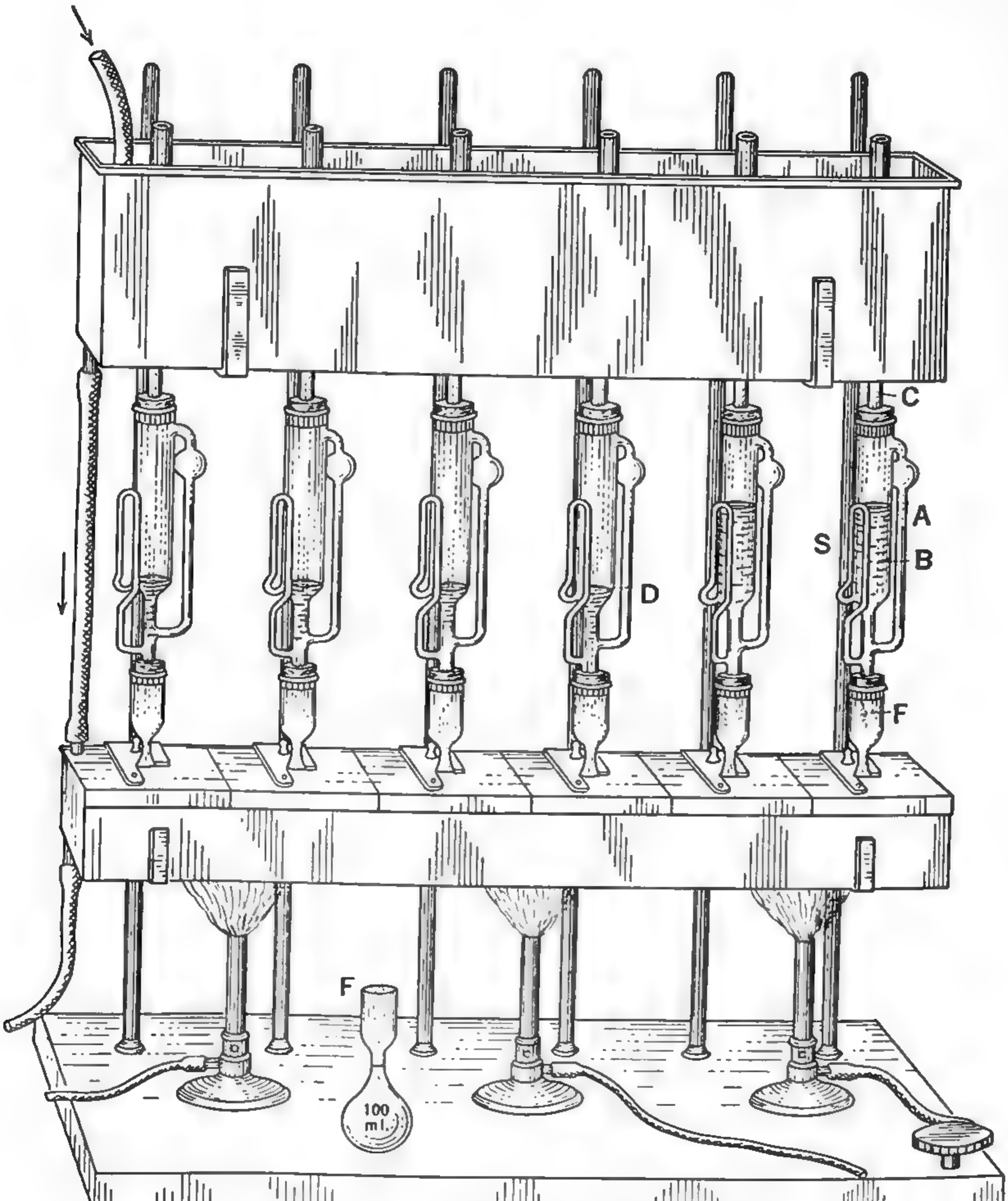


FIG. 187. Apparatus for Scheibler's alcohol-extraction method.

in *C* drops back upon the pulp in *B*. As soon as the level of the alcohol in *B* rises above the bend of the tube *S*, the alcoholic solution of sugar siphons mechanically into the flask *F*. The heat supplying

the water bath is regulated so that this siphoning occurs at least every 5 minutes, better every 3 to 4 minutes. The distilling and siphoning are continued until all the sugar is extracted, which, according to the fineness of the pulp, usually requires from 1 to 2 hours. If the pulp is so fine as to cause sluggish extraction, the process may be hastened by connecting the top of the condenser with an aspirator provided with a screw clamp and cautiously withdrawing air from the apparatus. (See Fig. 188.) This creates a slight vacuum and causes the alcohol to boil more vigorously. Immediately after the last siphoning the flask *F* is disconnected, cooled to 20° C., the volume completed to 100 ml. with 90 per cent alcohol, and the solution mixed, filtered, and polarized in a 200-ml. tube. The reading gives directly the percentage of sugar in the pulp. The extraction may also be performed with a double-normal weight of pulp, and correspondingly more subacetate solution. In this case it is advisable, however, to use a 200-ml. extraction flask and to double the amount of alcohol also.

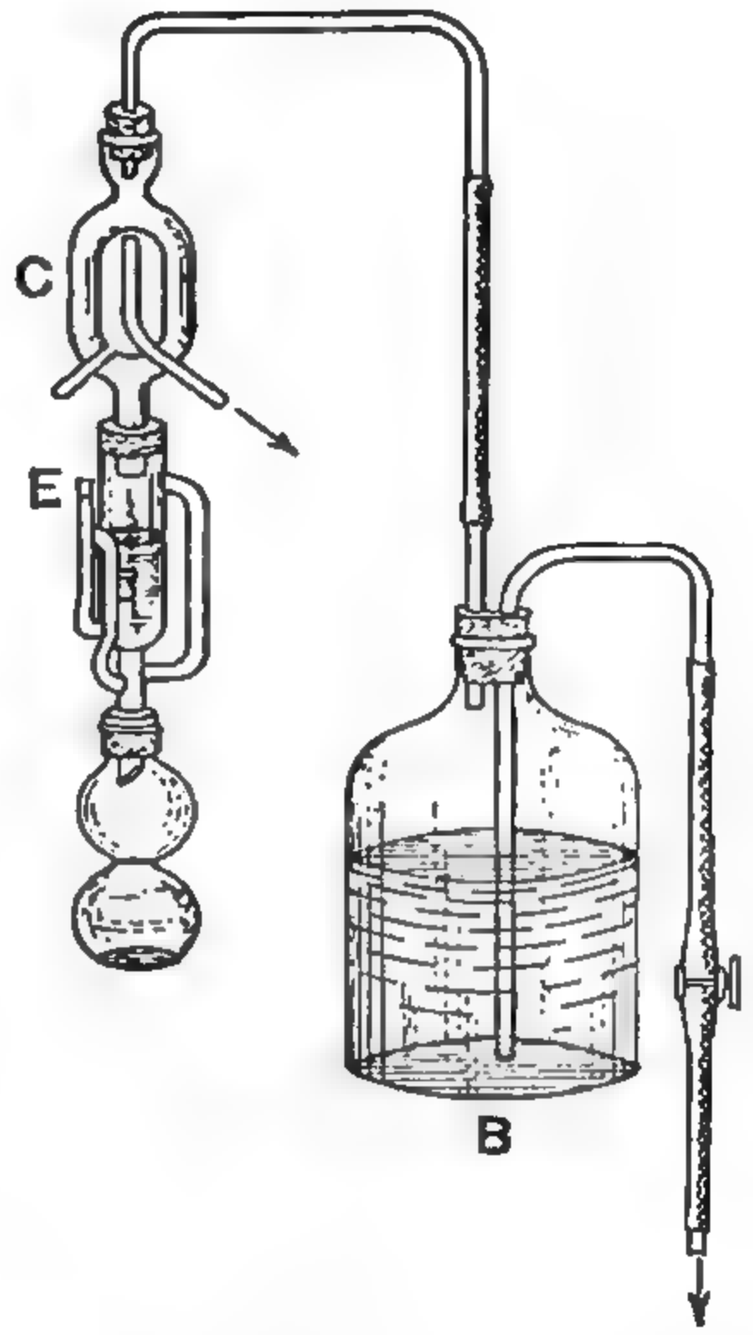


FIG. 188. Herzfeld's apparatus for alcoholic extraction under reduced pressure.

A form of extractor devised by Müller (Fig. 189) permits the withdrawal of a small quantity of liquid from the siphon tube for determining the completion of extraction. During operation the opening *a* is closed with a stopper. To obtain the sample this stopper is removed and a little of the liquid is sucked up with a pipette and subjected to the α-naphthol test (p. 659). If the test is positive, the stopper is replaced and the extraction continued until the reagent gives no coloration.

In determining sugar by the Scheibler process of extraction special care must be exercised to prevent evaporation of alcohol during filtration. The funnel should be covered with a watch glass and the filtrate received in a cylinder or flask with narrow neck. The first 20 to 30 ml. of the runnings should be discarded. The greater susceptibility of alcoholic sugar solutions to expansion and contraction with changes in heat and cold necessitates the maintenance of uniform temperature conditions during the polarization. The specific rotation of sucrose in ethyl alcohol is slightly higher (0.1° to 0.2°) than in water; but

the difference is so small that it falls within the limits of experimental error.

The method of alcoholic extraction gives results considerably lower than those calculated from the polarization of the expressed juice. The results of Scheibler previously quoted (p. 350) show a difference of about 0.75 for the polarization of sugar beets.

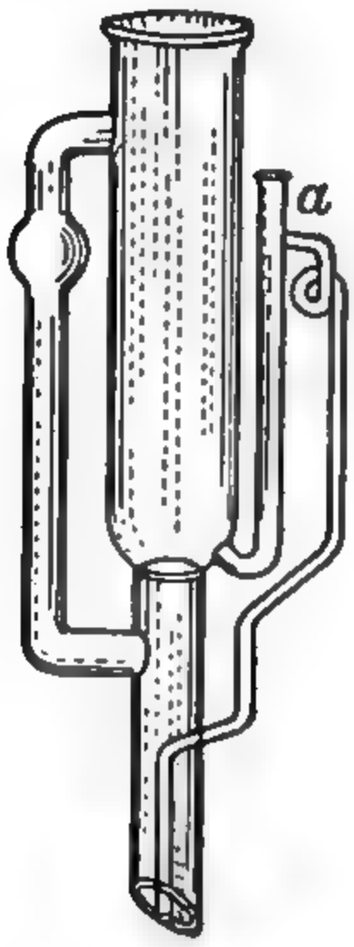


FIG. 189. Müller's modification of Soxhlet extractor.

Some authorities prefer adding the lead subacetate to the alcoholic extract rather than to the pulp previous to extraction. This practice is attended with some danger, however. One main object of adding the basic lead to the pulp is to neutralize any free acid which would otherwise invert some of the sucrose in the hot solution. In the presence of alcohol, lead subacetate solution must be used in the lowest possible amount owing to the danger of precipitating sucrose or of changing its specific rotation through formation of lead saccharate.

The alcohol-extraction method can be applied to the polarization of fruits and all other sugar-containing plant substances. With very dry materials the strength of the alcohol should be correspondingly reduced. With substances containing reducing sugars in large amount, it is desirable to omit the addition of lead subacetate, but when this is done the substance should be well mixed with powdered calcium carbonate to neutralize any free acid that might cause inversion.

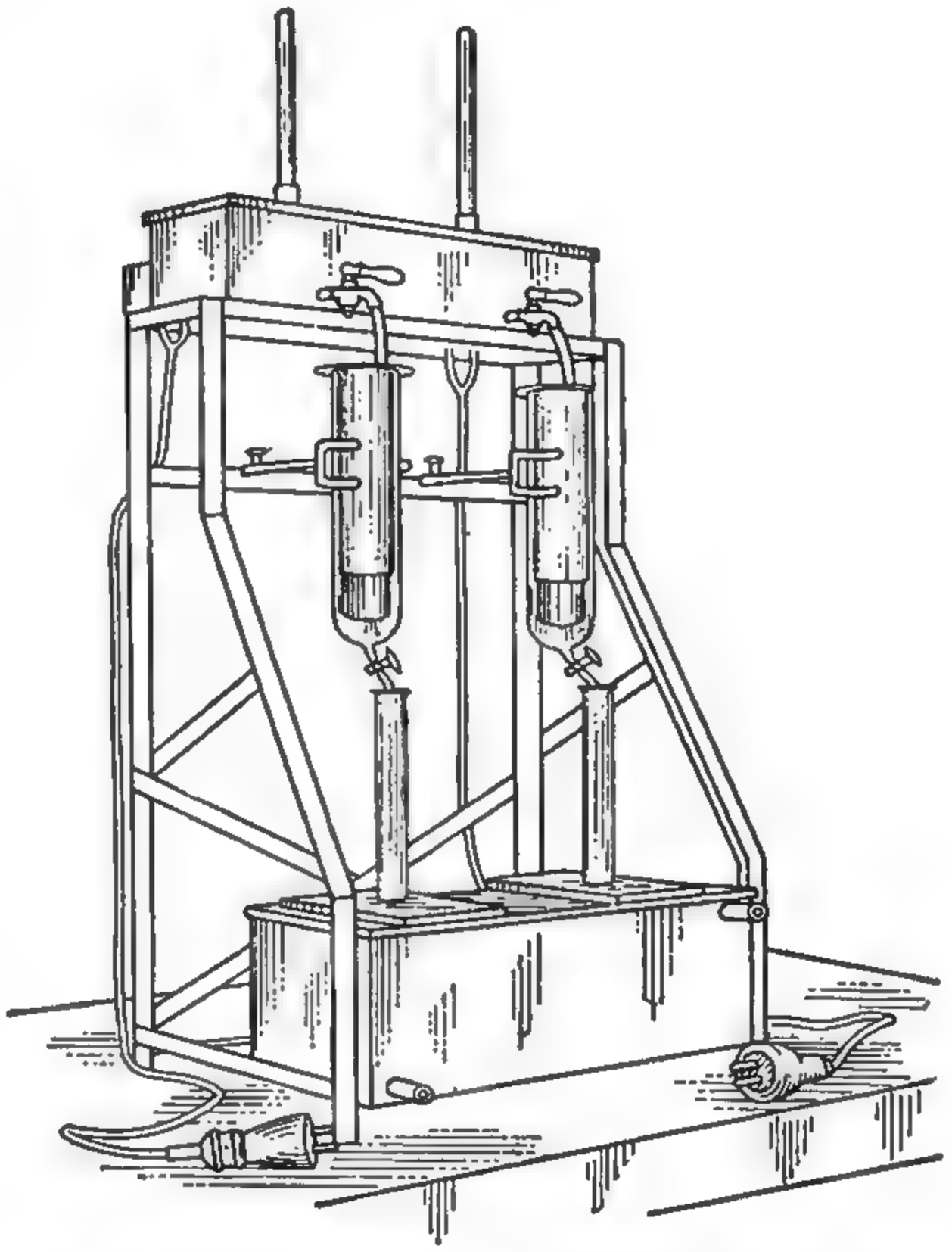
The alcohol-extraction method does not occupy the same position as a standard procedure that it formerly did. Herzfeld admitted that its absolute accuracy had not been proved. The principal sources of error are the effect of the alcohol on the polarization of the non-sugars and the effects of the excess lead and the acetates formed during clarification and of the prolonged heating on the rotation of sugar and non-sugars. Dolínek[101] concluded from experiments along this line that the combined errors may readily exceed 0.2 to 0.3 per cent sugar. Staněk and Vondrák[102] advocate removal of the alcohol from the extract by distillation, and polarization in aqueous solution, and also determination of sucrose by double polarization. But even this procedure would not obviate all the errors named. The use of the alcohol-extraction method as a standard for judging other methods has been abandoned in Czechoslovakia and other countries.

[101] *Z. Zuckerind. čechoslovak. Rep.*, **51**, 499 (1926/27).
[102] *Z. Zuckerind. čechoslovak. Rep.*, **51**, 51, 113 (1926/27).

IIB. Determination of Sugar in Plant Substances by Extraction with Water

If water is used instead of alcohol in extracting sugar for the polarization of plant substances, a process of percolation is preferable to reflux distillation because of the danger of decomposition through the prolonged heating of aqueous extracts. As an example of the water-extraction process the Zamaron method for determining sugar in sugar cane or in sugar beets is given.

Zamaron's Water-Extraction Apparatus. This apparatus,[103] Fig. 190, is a modification of an older design.[104] The extractor consists of a cylinder of aluminum or copper, to the lower end of which a basket of brass gauze is attached by means of a bayonet lock. This extractor fits loosely in an outer shell of Pyrex glass, with a glass stopcock of 2-mm. bore at the bottom. An electrically heated copper water bath, with constant level and thermometer, is placed above and a little back of the extractor. It is provided with a brass stopcock and a rubber tube through which the hot water flows into the extractor. The extract is collected in a 1500-ml. flask immersed in a bath of circulating cold water and held in position by a lead plate with a hole in the center. Two or more extractors may be mounted together on one stand, battery fashion. Before the beginning of the extraction 20 ml. of lead subacetate solution is measured into the flask; 100 g. of finely divided pulp is transferred to the basket through a metal funnel, and the boiling-hot water is

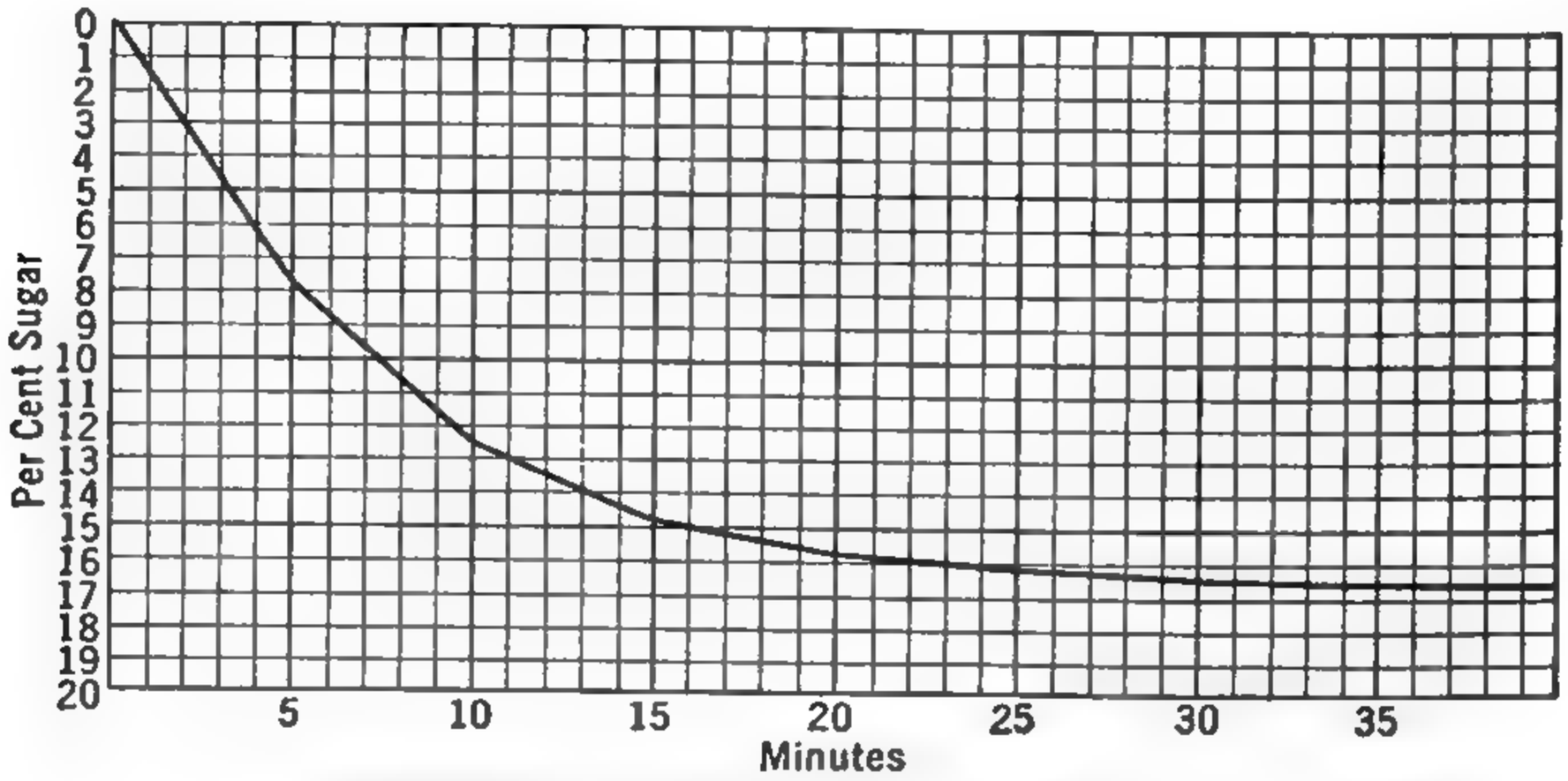


(Reproduced with permission from Bull. assoc. chim., 55, 462.)

Fig. 190. Zamaron's apparatus for hot-water extraction.

[103] *Bull. assoc. chim.*, 55, 462 (1938).
[104] *Bull. assoc. chim. sucr. dist.*, 15, 74 (1897/98).

turned on, the flow being regulated so that a constant level is maintained in the extractor and the flask is filled nearly to the mark in 30 to 35 minutes. The volume is completed at 20° C.; the solution is shaken, filtered, and polarized in a 400-mm. tube. The reading, multiplied by 1.95 (i.e., 1.3 × 1.5), gives the polarization (degrees Ventzke) of the pulp.



(Reproduced with permission from Bull. assoc. chim., 55, 462.)

FIG. 191. Curve showing course of extraction by Zamaron's method.

Zamaron's experiments showed that 88 per cent of the sugar is extracted in the first 15 minutes, and that the last portions of the extract are free from sugar. The course of the extraction is shown in the curve of Fig. 191. The method gave practically the same results as Pellet's hot aqueous digestion method.

The extraction may also be effected intermittently, by first pouring 250 ml. of the boiling-hot water on the pulp, the lower stopcock on the extractor being closed. After 1 minute the stopcock is opened and the flow regulated so that the extract runs through in 4 to 5 minutes. After closing the cock again 200 ml. of water is poured on the pulp in the same manner as before, and the operation is repeated until nearly 1500 ml. of extract has been collected. The results are the same as those obtained by continuous percolation.

The principal objection against the Zamaron process is the danger of incomplete extraction. This error may be reduced by using only 50 g. of pulp for the extraction, as recommended by Fribourg,[105] but this procedure, while halving the errors of extraction, necessitates a doubling of any error in the polariscope reading.

Another source of error in the method of hot-water extraction described is the danger of inversion of sucrose through the natural acidity of the pulp. One method of preventing this is to mix with the pulp

[105] Fribourg's "Analyse chimique," p. 223.

previous to extraction finely powdered calcium carbonate. Another method[105] is to employ very dilute milk of limewater for the extraction. The presence of minute quantities of free alkali does not affect the determination of sucrose; a danger exists, however, in the action of hot alkaline solutions (even when very dilute) in modifying or destroying reducing sugars. Careful neutralization of the free acid in the pulp with limewater or dilute sodium carbonate solution would eliminate the risk of inversion without serious danger of affecting the reducing sugars.

The volume error caused by the lead precipitate may be obviated by adding the lead subacetate to the pulp or to the water used for the extraction; this will also provide the necessary alkalinity, but it may also retard the extraction, as claimed by Martraire.

Another objection to the method of hot-water extraction is the solution of optically active gums, hemicelluloses, and pectic acid. These substances introduce at times a considerable error in the polarimetric determination of sugars in aqueous plant extracts. This error does not exist in the alcohol-extraction method, owing to the insolubility of these substances in ethyl alcohol. However, according to Pellet, they are precipitated by lead subacetate, and if a sufficient quantity of this clarifying agent is used they do not interfere.

Steuerwald[106] has shown that neither the aqueous nor the alcoholic extraction method gives correct results upon sugar cane or bagasse, and that it is preferable to calculate the sugar content from other analytical data.

III. Determination of Sugar in Sugar Beets by Methods of Digestion

The method of alcoholic extraction, although still considered by some to be the most accurate, is not the best from a practical standpoint on account of the long period of time necessary for extraction, and also because of the rather fragile nature of the extraction apparatus. For the rapid determination of sucrose in sugar beets some one of the numerous digestion processes is usually followed.

The digestion method may be regarded in principle as a combination of the extraction and juice-expression methods. A weighed amount of pulp is digested with a large excess of alcohol or water. After the complete diffusion of the sugar through the liquid, the solution is made up to volume, allowing for the space occupied by insoluble matter, and then filtered and polarized.

[106] *Arch. Suikerind.*, 21, 471 (1913).

Volume Occupied by the Marc. Although Heintz had shown as early as 1874 (see p. 350) that dry sugar-beet marc absorbs water from sugar solutions, and Scheibler a few years later demonstrated the presence of colloid water in the fresh sugar-beet marc, the correction for the volume of the marc in the digestion methods of sugar determination was nevertheless based for decades on the weight and specific gravity of the dried marc. The figure generally accepted was that of Rapp and Degener.[107] An average dry marc content of 4.8 per cent in the beet was assumed, or approximately 1.2 g. in the normal weight of 26 g., and the density of the marc was taken as 2.0, resulting in $1.2 \div 2$, or 0.6 ml. for the volume of the marc. Other authorities have given 0.75 ml.,[108] 0.8 ml.,[109] or 1.35 ml.[110] The correction for the volume of the marc may be made by decreasing the normal weight and keeping the volume of the flask constant, but the preferred procedure is to adhere to the normal weight of 26 g. and to increase the volume of the flask. Pellet devised a special digestion flask with five graduations at 200.0, 200.5, 200.75, 201.0, and 201.5 ml., so that the chemist may vary the volume according to the weight and character of the marc.

In 1895 Scheibler[111] confirmed his original findings about the presence of colloid water in the marc by his double dilution method (p. 313) and also by an independent method. The average result was 2.5 ml. volume for the hydrated marc from the normal weight of the beet, much higher than that occupied by the dry marc.

A novel procedure for the determination of the hydrated marc was employed by Spengler and Brendel.[112] The dry marc was determined by the method of Claassen (p. 348). Another, 100-g., portion of the same beet pulp was also extracted by Claassen's method, and the wet marc transferred to a 500-ml. tared Erlenmeyer flask. Enough water was added to raise the weight of the flask contents to about 100 g., and the weight was accurately determined. Then 50 ml. of 0.1 *N* thiosulfate solution was added; this diluted only the free water adhering to the pulp, but not the colloid water. The flask was stoppered and allowed to stand for 10 minutes with frequent shaking. The mixture was then pressed out in a flannel bag and placed in a funnel, until a little over 100 ml. of filtrate was obtained. Exactly 100 ml. was pipetted out and titrated with 0.1 *N* iodine solution.

107 *Z. Ver. deut. Zucker-Ind.*, 32, 786 (1882).
108 Fribourg's "Analyse chimique," p. 253.
109 Pellet, *Z. Ver. deut. Zucker-Ind.*, 56, 903 (1906).
110 Sidersky's "Manuel," p. 241.
111 *Neue Zeitschrift*, 34, 69, 127, 140 (1895).
112 *Z. Ver. deut. Zucker-Ind.*, 76, 880 (1926).

In a typical experiment the hydrated marc and adhering water from 100 g. of beet pulp weighed 102.33 g. After treatment with thiosulfate, the 100 ml. of filtrate required 35.0 ml. iodine solution for the titration. The solution therefore consisted of 35.0 ml. 0.1 *N* thiosulfate solution and 65 ml. of free water adhering to the marc hydrate. The added 50 ml. of thiosulfate solution corresponded to $50 \times 65 \div 35$, or 92.86 ml. of free water. Hence the amount of marc hydrate was $102.33 - 92.86 = 9.47$ g., or 9.47 per cent on the weight of beet pulp. In another experiment a sodium chloride solution was used instead of the thiosulfate solution, and titrated with silver nitrate. The result was 9.18 per cent marc hydrate. The average of the two determinations, 9.3 per cent, checks closely with Scheibler's figure, 9.66 per cent.

Thirteen beet samples, tested during one campaign by this method, gave 8.2 to 11.2, average 9.3, per cent marc hydrate, and 3.0 to 4.5, average 3.5, per cent dry marc of an average density of 1.13. The normal weight of beet pulp contains therefore $9.3 \times 26 \div 100$, or 2.42 g. marc hydrate, occupying a volume of $2.42 \div 1.13$, or 2.1 ml., over three times as much as the 0.6 ml. previously used for the correction, on the basis of dry marc. Somewhat lower figures were obtained by Müller and Pucherna[113] (1.7 ml.), and by Kopecký[114] (1.3 ml.). For beets grown in Southern California Bachler[115] found 2.31 ml.

Effect of Lead Subacetate on the Volume of the Marc Hydrate. In actual practice the beet pulp is usually digested not with pure water or alcohol but with lead subacetate solution which is added for clarification. A systematic investigation by Staněk and Vondrák[116] demonstrated that the marc hydrate in the presence of lead subacetate occupies less volume than the marc hydrate itself. These authors digested the normal or double-normal weight of beet pulp, after addition of the usual amount of lead subacetate solution, with hot water in a 200-ml. flask, and polarized the digestion filtrate. The remaining marc was repeatedly washed with water and centrifuged. After the sugar had been completely removed the entire filtrate and washings, including the liquid used for the polarization, were evaporated in a high vacuum to a small volume, made up to 200 ml., and polarized. The volume of the marc hydrate plus lead precipitate was then calculated from the apparent and true polarization. The average obtained during three beet campaigns was 1.54 ml. for the normal weight of beet pulp. Saillard[117] reported a volume of 1.2 to 1.3 ml. for the

[113] *Z. Zuckerind. čechoslovak. Rep.*, **54**, 99 (1929/30).
[114] *Z. Ver. deut. Zucker-Ind.*, **81**, 447 (1931).
[115] *Facts About Sugar*, **29**, 191 (1934).
[116] *Z. Zuckerind. čechoslovak. Rep.*, **51**, 101, 113 (1926/27).
[117] *Circ. hebd. fabr. sucre*, June 19 and 26, July 3 and 24 (1932).

double French normal weight, corresponding to 1 ml. for the normal weight of 26 g.; Spengler and Paar[118] found 0.83 ml., Osborn[119] 1.0 ml., both for 26 g. of beet. All these figures are lower than that for the marc hydrate itself, and it is concluded from this that the added lead subacetate causes the marc hydrate to shrink by withdrawing colloid water.

In a later investigation Spengler, Paar, and Mück[120] found an average of 4.8 per cent of dry marc in the beet, a volume of 2.1 ml. for the marc hydrate in 26 g. of beet pulp, and a volume of 1.06 ml. for the marc hydrate in the presence of lead subacetate. An increase in the amount of lead over that required for clarification causes a further shrinkage of the marc hydrate.

The volume of the marc hydrate plus lead precipitate naturally varies with the nature of the beet, and for this reason the International Commission for Uniform Methods of Sugar Analysis at its Eighth Session, held in 1932, decided not to recommend a definite volume correction, but to leave this matter to individual countries or districts. The value of 1 ml., which is the average of the figures reported by Osborn, by Spengler, Paar, and Mück, and by Saillard, has been adopted in the United States and in Germany, but in Czechoslovakia the value found by Staněk and Vondrák, 1.5 ml., is employed for the volume correction.

Determination of the Colloid Water. Spengler, Paar, and Mück have calculated the colloid water *Ko* in the marc hydrate plus lead precipitate by the following formula:

$$Ko = G - c(V - v) - g$$

where V is the volume of the flask, G the weight of its contents, v the volume of the hydrated marc and lead precipitate, g the weight of the dry marc plus lead precipitate, and c the specific gravity of the digestion liquid. Beets with 3.96 per cent dry marc were found to contain 0.12 g. colloid water per normal weight, beets with 5.47 per cent marc 0.20 g. The normal weight of average beets with 4 to 5 per cent marc may therefore be assumed to contain 0.15 g., or 0.15 ml. colloid water.

Adsorption of Sucrose by the Marc. Martraire[121] has found that when beet pulp is analyzed by hot aqueous digestion, and a known amount of sucrose added to another portion of the same pulp, the

[118] *Z. Ver. deut. Zucker-Ind.*, **83**, 342 (1933).
[119] *Ind. Eng. Chem., Anal. Ed.*, **6**, 37 (1934).
[120] *Z. Ver. deut. Zucker-Ind.*, **87**, 594 (1937).
[121] *Bull. assoc. chim.*, **52**, 775 (1935); **53**, 609, 617 (1936); **55**, 441 (1938).

sugar recovered in the second digestion is less than the sum of that recovered in the first digestion and the sucrose added in the second. This and similar results obtained in other experiments are ascribed by Martraire to adsorption of sucrose by the marc. The same explanation had previously been offered by Pellet and others. But the phenomenon may be explained also by a dehydrating effect of sucrose on the marc, as suggested by Spengler and Paar. It is possible, of course, that both adsorption and dehydration play a part, and this subject requires further investigation.

Alcohol-Digestion Methods. The first process of digestion employed alcohol, and is known as the Rapp-Degener[122] method. The procedure was similar to that of Pellet's hot aqueous-digestion method described below, but a reflux condenser was used to prevent loss of alcohol. A cold alcohol-digestion method, analogous to Pellet's cold aqueous-digestion method, was developed by Stammer.[123] These alcohol-digestion methods were found to give unreliable results, usually too low, because either the sugar was not completely extracted or was partly precipitated by excess lead subacetate in the presence of alcohol. For this reason, and also because alcohol is too expensive for routine work, the alcohol-digestion methods have been entirely abandoned in favor of the aqueous methods.

The aqueous-digestion methods may be subdivided into three classes: (1) methods in which the normal weight of pulp is made up with water to a definite volume in a flask (method of Pellet and modifications); (2) methods in which a definite volume of water is added from a pipette to the normal weight of pulp (method of Sachs-Le Docte and modifications); (3) Krüger's method, in which pulp and water are mixed in definite proportions, but a normal weight is not required.

Pellet's Hot-Water-Digestion Process. This method, devised by Pellet[124] in 1887 and at about the same time by Herles[125] has been variously modified. It does not require pulp of extreme fineness, but samples obtained by the use of a meat grinder or similar apparatus are satisfactory. As an example, the procedure used by the Great Western Sugar Company[126] is described:

Weigh out 26 g. of the well-mixed sample as rapidly as possible, and rinse with a jet of water, using about 100 ml., into a 201.0-ml. Kohlrausch (or Stift) flask, Fig. 192. Place under a good vacuum for 5 to 10 minutes to remove

[122] *Z. Ver. deut. Zucker-Ind.*, 32, 514, 786, 861 (1882); 34, 1366 (1884).
[123] *Z. Ver. deut. Zucker-Ind.*, 33, 206 (1883).
[124] *Neue Zeitschrift*, 19, 315 (1887).
[125] *Z. Zuckerind. Böhmen*, 11, 531 (1886/87).
[126] Private communication from S. J. Osborn.

air, being careful to avoid mechanical loss when the vacuum is first applied. Then add sufficient water to make a volume of about 175 ml., and digest in a water bath at 80° C., supporting the flask so that its body is entirely immersed but is not in contact with the heating coils at the bottom of the bath. Two or three times during the digestion period remove the flask, mix by swirling, and wash down the pulp that adheres to the neck or walls of the flask with a little water at 80° C. after each agitation. After exactly 30 minutes' digestion fill to within 2 to 5 ml. of the mark with water at 80° C. and continue the digestion for exactly 10 minutes longer. This second digestion is important to equalize concentrations, as pointed out by Weisberg.[127] Cool to approximately room temperature in a cold-water bath, remove the flask from the bath and allow to stand 5 to 10 minutes to bring the solution to room temperature, then add 6 ml. of basic lead acetate and the necessary small amount of water at room temperature to bring to the mark. (The dilution made during the hot digestion should be such that only a small amount, not over 4 ml., of water is required for the completion of the final volume.) Mix the contents of the flask well by shaking, allow to stand 5 minutes to insure concentration equilibrium, then shake again and filter. Polarize in a 400-mm. glass tube, equipped with slip caps or Ninegar caps (p. 244), allowing the solution to stand in the tube in the polariscope box for at least 5 minutes before reading. The reading gives directly the percentage of sugar.

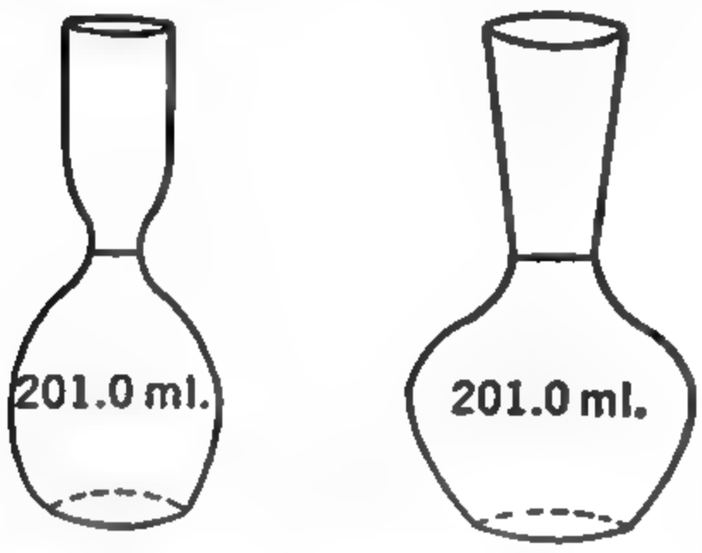


FIG. 192. Flasks for digestion of beet pulp.

If trouble is experienced with foam, the flask may be put under vacuum a second time after cooling, or a few drops of ether or 1 drop of amyl alcohol may be added either just before the second digestion period or just before the completion of the final volume.

Beets of abnormally low purity may require 8 to 10 ml. of basic lead subacetate solution for clarification. The digestion bath should be equipped with two accurate thermometers, to check against each other in case either should develop any inaccuracy.

In the Pellet hot-water-digestion method as carried out in Germany,[128] and in the Herles[129] modification practiced in Czechoslovakia, the lead subacetate solution is added before the digestion, and there are some other minor differences in conditions and manipulation from the procedure just described. In the Herles method the flask volume is increased 1.5 ml. instead of 1.0 ml., for each normal weight (see p. 362).

[127] *Bull. assoc. chim. sucr. dist.*, **25**, 600 (1907/08).
[128] Frühling's "Anleitung," 10th ed. by Spengler, p. 190, 1932.
[129] *Z. Zuckerind. čechoslovak. Rep.*, **59**, 43 (1934/35).

Osborn found that in the analysis of normal beets the lead subacetate solution may be added before the digestion, but on decomposed beets there was some indication that with this procedure the polarizations were too high.

Pellet's Cold-Water-Digestion Process.[130] To obtain reliable results by this method, the beets or cossettes must be reduced to an extremely fine pulp, in the Sans-Pareille or Herles press (pp. 342–345). Four to six milliliters of lead subacetate solution is placed in a 201.0-ml. Kohlrausch flask, and 26 g. of beet pulp is washed into the flask with sufficient water to fill the flask about two-thirds. The flask is well shaken, and a little ether is added from time to time to break the foam. Water is then added nearly to the mark, the foam destroyed with a drop of ether, and the volume completed. The solution is well mixed, filtered, and polarized in a 400-mm. tube, the reading giving directly the percentage of sugar. The entire operation requires only a few minutes, but there is always some danger of incomplete extraction. This method has been entirely superseded by the Sachs-Le Docte or the Krüger method, both of which are more convenient.

Sachs-Le Docte Process of Water Digestion. The occlusion of air bubbles by the pulp and the uncertainty of knowing whether such bubbles are completely absent before making up to volume have been the principal objections against the Pellet process of digestion in a volumetric flask. To meet this difficulty, Kaiser and Löwenberg[131] proposed in 1892 to add to the normal weight of pulp a constant volume of water and lead subacetate solution so that the final estimated volume of solution, regardless of insoluble marc or occluded air, is always 100 ml. This idea was further elaborated by Sachs and Le Docte.[132] The total volume was increased to 200 ml., in order to insure complete extraction and perfect admixture, and to obtain more filtrate for the polarization. The volume of water and lead subacetate solution to be added was calculated to equal 177 ml., derived from the following consideration. Sachs assumed as the average marc and juice content of the sugar beet 4.75 per cent and 95.25 per cent, respectively. For the normal weight (26 g.) of pulp there would then be 26 g. $\times$ 0.9525 = 24.765 g. juice. The average sugar content and density of juices from beets of different richness are given in Table LX together with the calculated volume of juice (24.765 $\div$ sp. gr.), the

[130] *Deut. Zuckerind.*, 13, 1229 (1888); 15, 531 (1889); Sidersky's "Manuel," p. 241.
[131] See Le Docte, *Intern. Sugar J.*, 29, 214 (1927).
[132] Published in pamphlet form by Le Docte, 1895; *Intern. Sugar J.*, 29, 488 (1927).

volume of lead-water solution (200 ml. less the volume of juice) and the polarization error resulting from use of the constant volume 177 ml.

TABLE LX

Sugar in Beet	Sugar in Juice	Brix of Juice	Specific Gravity of Juice	Volume of Juice	Volume of Lead-Water Solution	Calculated Polarization*	Polarization Error
per cent	per cent			ml.			
12	12.59	14.86	1.0609	23.34	176.66	11.979	−0.021
13	13.65	15.82	1.0651	23.25	176.75	12.984	−0.016
14	14.70	16.82	1.0694	23.16	176.84	13.988	−0.012
15	15.75	17.86	1.0740	23.06	176.94	14.995	−0.005
16	16.80	18.92	1.0787	22.96	177.04	16.003	+0.003
17	17.85	20.00	1.0835	22.86	177.14	17.012	+0.012

* Calculated polarization $= \frac{\text{sugar in beet} \times 200}{\text{volume of juice} + 177}$.

It is seen that by use of the constant volume 177 ml. the calculated polarization error is too small to be detected upon the saccharimeter.

The constant-volume pipette employed in the Sachs-Le Docte process is shown in Fig. 193. A three-way cock *K* at the bottom serves for the inlet of lead reagent and water at *B* and *C* and for the delivery of the 177 ml. of mixed solution through *D*. The cap *A* at the top, which receives the overflow, is connected with a waste bottle. Instead of drawing in the lead reagent and water separately, a single " lead-water " solution of proper dilution may be used. One of the cock connections may thus be dispensed with. By raising or lowering the capillary tube *h* upon its support at *H* the capacity of the pipette is easily adjusted to exactly 177 ml.

A more modern modification of the Sachs-Le Docte pipette is that of Richter, Fig. 194.

A pipette of the form shown in Fig. 196 (Krüger pipette), adjusted to 177 ml. of lead water, may also be used.

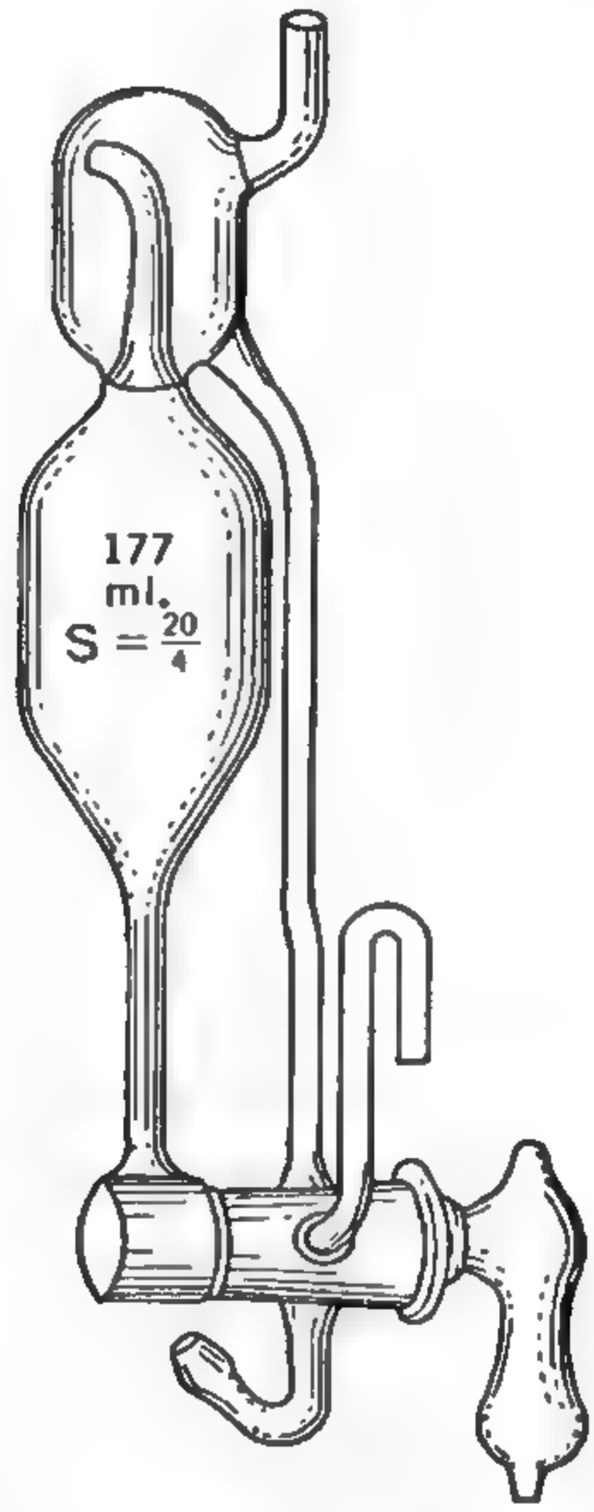
Staněk and Vondrák[133] found an average juice content of only 21.8, instead of 23.0 ml., for the normal weight of pulp, and consequently the official methods used in Czechoslovakia prescribed for some years a pipette volume of 356.4 (178.2 × 2) ml. for the double-normal weight of pulp. With the introduction of the new normal weight of 26.026 g., the volume has been reduced to 356.35 ml. Spengler, Paar, and Mück[134] have shown that for German beets, under the conditions of the Sachs-Le Docte method, the colloid water in the hydrated marc

[133] *Z. Zuckerind. čechoslovak. Rep.*, 51, 101, 113 (1926/27).
[134] *Z. Ver. deut. Zucker-Ind.*, 87, 594 (1937).

and lead precipitate averages 0.15 ml. per normal weight of pulp. This must be deducted from the juice volume of 23 ml., and the pipette should therefore be calibrated to hold 177.15 ml. The original 177-ml. pipette is still the one most widely used, but for greater accuracy the volume should be adjusted to suit the beets grown in a particular country or district.

The digestion was carried out originally in tinned brass "capsules," holding about 325 ml., and provided with a disk cover and rubber

FIG. 193. Sachs-Le Docte automatic pipette for sugar-beet analysis.



(Reproduced with permission from Frühling-Spengler, "Anleitung zu Untersuchungen," p. 189.)

FIG. 194. Richter's automatic pipette for sugar-beet analysis.

cap to seal the capsule hermetically. Similar capsules of Monel metal or nickel, with aluminum covers and rubber envelopes, are generally used in the United States. Herzfeld[135] introduced bottles of copper or nickel-plated iron, 11 cm. high, 6 cm. in body diameter, and with a neck 4 cm. in diameter and 2 cm. high. These bottles are closed with a well-fitting cork or rubber stopper, covered with tin foil. A disadvantage of these bottles is that the stopper may blow out if the

[135] *Z. Ver. deut. Zucker-Ind.*, 59, 627 (1909).

hot-digestion process is used. Spengler[136] recommends lacquered friction top cans, about 12 cm. high by 7 cm. in diameter; Staněk and Urban,[137] small milk cans of tinned sheet steel, with a spring cap and rubber gasket. For serial analyses in large numbers it is advisable to adjust all the capsules or other receptacles to the same tare, so that they may be interchanged.

The normal weight of very fine pulp, prepared with the Keil-Dolle rasp, or the Sans-Pareille or Herles press, is weighed out rapidly on a tared metal scoop, or on a counterpoised piece of parchment or glazed onion-skin paper, about 4 by 4 inches. The scoop or paper with the pulp is transferred to a capsule, and 177 ml. of lead water (1 volume of lead subacetate solution and 30 to 40 volumes of water) is added from the pipette. The cover is put on, the capsule shaken vigorously for a few seconds, and the contents filtered and polarized in a 400-mm. tube. The reading gives the percentage of sugar directly. As a further precaution, the methods of the Great Western Sugar Company specify that after the first shaking the capsule be allowed to stand for at least 20 minutes, and again shaken vigorously before the filtration.

If the particles of pulp are not fine enough for cold-water digestion, the closed capsule or bottle, after being vigorously shaken, is placed for 30 minutes in a water bath at 75 to 85° C., and then cooled to 20° C. It is again well shaken when the contents are filtered and polarized in the usual way.

Bachler's Modification of the Sachs-Le Docte Method.[138] Bachler digests the beet pulp with water alone, and then clarifies with Horne's dry subacetate of lead. This makes it possible to determine both the soluble solids and the sugar upon the same sample. In this method the volume of the juice must be derived from the marc hydrate, not from the mixture of marc hydrate and lead precipitate. Bachler found in the beets produced in Southern California an average juice volume of 20.9 ml., and therefore adds 179.1 ml.[139] water to the normal weight of pulp. After the digestion the filtrate is used first for the determination of the refractive index by means of the immersion refractometer with the Goldbach flow-through cell (p. 122). Dry lead subacetate is then added to the liquid, and the mixture is well shaken and polarized. The Pellet digestion method may also be employed, instead of the Sachs-Le Docte, with a flask corrected for the volume

[136] Frühling's "Anleitung," 10th ed., p. 188, 1932.
[137] *Z. Zuckerind. Böhmen,* **34,** 625 (1909/10).
[138] *Facts About Sugar,* **29,** 191 (1934).
[139] In a later publication, *Facts About Sugar,* **32,** 327 (1937), Bachler gives 178.4 ml.

occupied by the marc hydrate in the absence of lead subacetate (see p. 360).

Krüger's Cold-Water-Digestion Process. Shortly after the publication of the Sachs-Le Docte method, Krüger[140] devised another procedure, based on the idea of Kaiser and Löwenberg, but dispensing entirely with the use of normal weights. The principle of the method may be understood from the following.

The weight of juice per 26 g. in an average sugar beet of 5 per cent marc content is $26 \times 0.95 = 24.7$ g. The specific gravity of the average beet juice is very nearly 1.07, so that the volume of juice in a normal weight (26 g.) of pulp is 24.7 g. $\div$ 1.07 = 23.08 ml. The amount of water necessary to complete this volume of juice to 100 ml. is therefore 100 — 23.08 = 76.92 ml. The ratio of normal weight to volume of added water is then 26 g. : 76.92 ml. = 1 g. : 2.958 ml. If the figure of Spengler, Paar, and Mück, 22.85 ml. of juice in the normal weight of pulp, is accepted, the ratio of pulp to water is as 26 g. to 77.15 ml. = 1 g. to 2.967 ml., or in round numbers 1 g. : 3 ml. The addition, therefore, of water in the proportion of 3 ml. to every 1 g. of pulp yields a solution whose polarization in a 200-mm. tube will give the approximate sugar content of the beet.

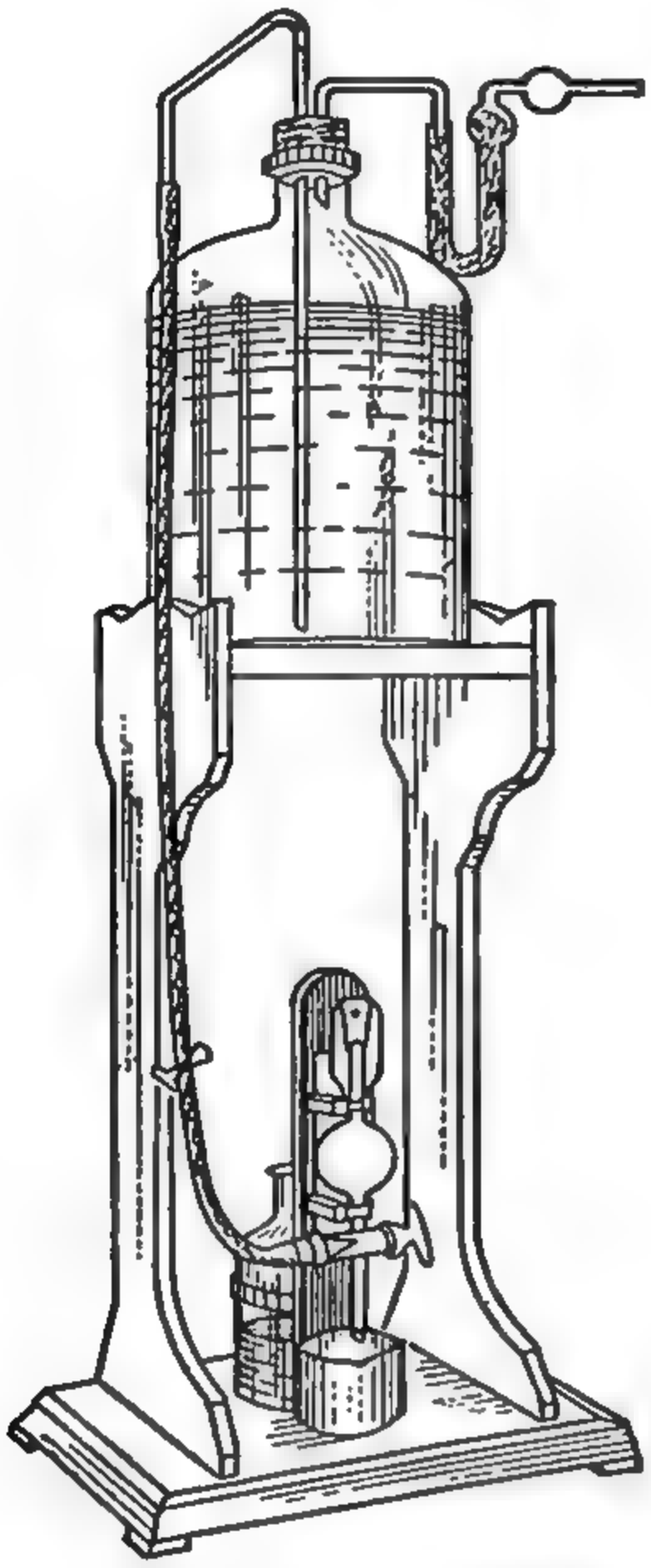
If it is desired to dilute the half-normal weight of pulp to 100 ml., instead of to 50 ml., the ratio of pulp to water is not as 1 g. to 6 ml., but as 1 g. to 6.8 ml. The volume of the juice then equals $13 \times 0.95 \div 1.07$, or 11.54 ml., and the water to be added is 88.46 ml., which is 6.8 times the weight of pulp in grams.

The automatic pipette designed by Frühling for the Krüger process is shown in Figs. 195 and 196. It is fastened to a fixed support and provided with a three-way stopcock. When the handle is in the horizontal position the lead water (25 ml. lead subacetate solution diluted with 1 liter of water) flows from the supply bottle through opening *a* in the stopcock into the pipette. Any excess runs through an overflow into bottle *b*. When the handle is turned to the vertical position the pipette empties into the dish below. The supply bottle is provided with a soda lime tube to prevent contamination with carbon dioxide.

The pipette is prepared in several sizes for approximately double-normal, normal, half-normal, and quarter-normal weights of pulp, i.e., approximately 50 g. (150 ml.), 25 g. (75 ml.), 12 g. (36 ml.), and 6 g. (18 ml.). The smaller sizes are used for polarizing the small pulp samples obtained with the Keil-Dolle boring machine, or for core samples.

[140] *Deut. Zuckerind.*, 21, 2434 (1896).

The weight of pulp corresponding to each pipette is determined by calibration with water, as in the following example. The weight of distilled water discharged by a Frühling pipette at 20° C. was found to be 78.38 g. The volume of the pipette in milliliters is then 78.38 ÷ 0.9972 = 78.6 ml.; 78.6 ÷ 3 = 26.2 g., or more exactly 78.6 ÷ 2.967 = 26.49 g., the weight of beet pulp corresponding to the pipette.



(*Reproduced from Frühling-Spengler, "Anleitung zu Untersuchungen," p. 187.*)

FIG. 195.

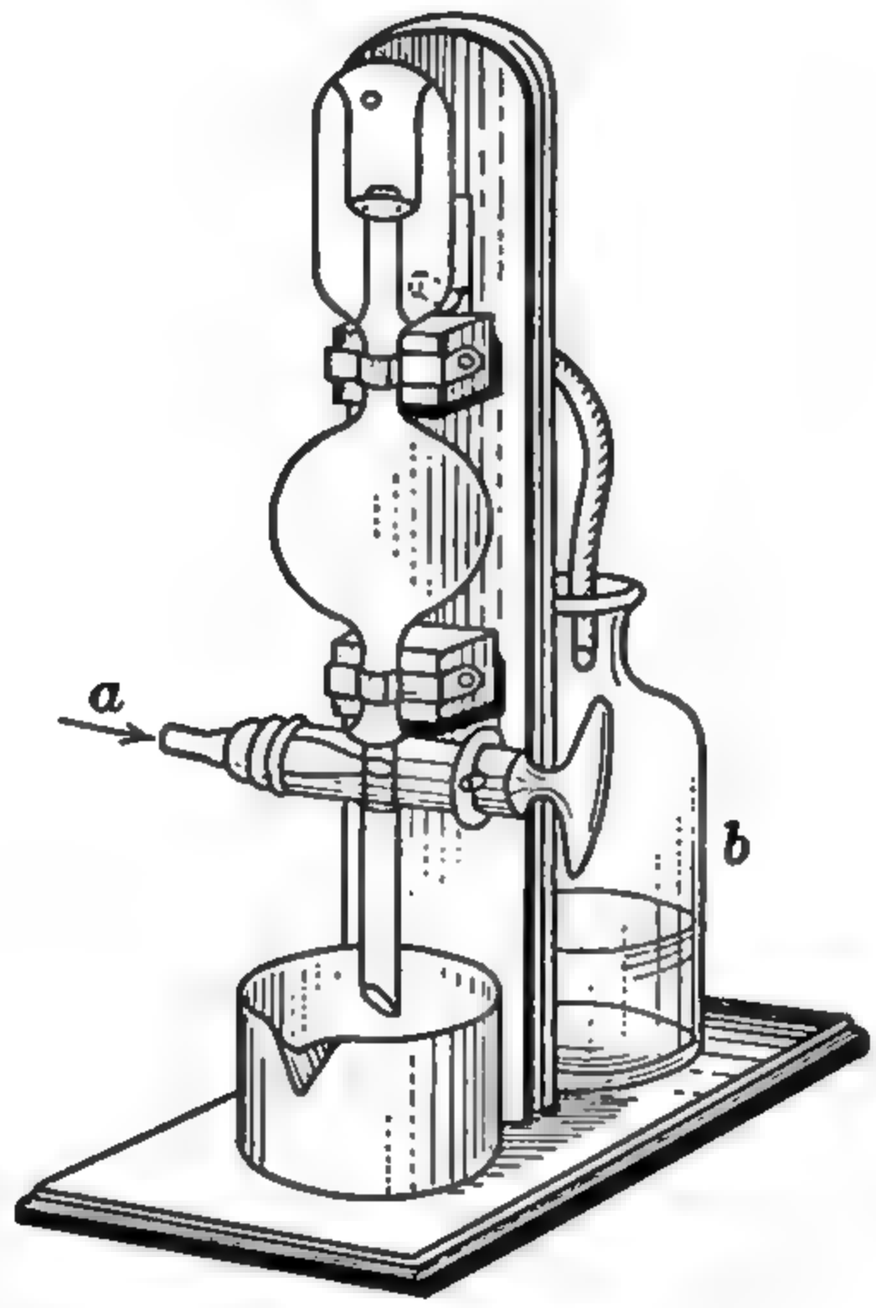


FIG. 196.

Frühling's pipette for Krüger's method of sugar-beet analysis.

The calculated quantity of pulp is weighed into the nickel dish, the lead water added from the pipette and well mixed with the pulp by stirring with a glass rod. After a few minutes the mixture is filtered, and the filtrate polarized in a 200-ml. tube.

The Krüger process has been criticized by Le Docte[141] on several grounds. The use of pipettes differing in volume is likely to lead to confusion and error when several pipettes are used in the same laboratory. Because of the small proportion of lead water used, only

[141] *Intern. Sugar J.*, 29, 214 (1927).

about 78 ml. against 177 ml. in the Sachs-Le Docte method, the polarization errors are much greater when the sugar content of the pulp differs appreciably from the normal. Stirring with a glass rod does not assure perfect admixture and complete extraction in the short time within which the process must be finished to avoid evaporation. The quantity of filtrate obtained is frequently insufficient for polarization with the Pellet continuous tube. For these reasons the Krüger method has been abandoned in most countries, and is only used to a certain extent in Germany.

Cold-Digestion Method of Staněk and Pavlas for Large Samples of Fresh Cossettes. In order to avoid the error caused by loss of moisture when fresh cossettes are prepared for analysis, Staněk and Pavlas[142] have devised a machine by which a large sample (1252 g.) of cossettes can be ground in 5 minutes to a very fine pulp in the presence of a large quantity (8570 ml.) of water in a hermetically sealed container, the sugar being extracted while the sample is being ground. Ten milliliters of benzene is added to the water, to destroy the protoplasm and facilitate the extraction. A sample of the mixture is withdrawn from the container, clarified with dry subacetate of lead, filtered, and the filtrate polarized in a 400-mm. tube. The reading gives directly per cent sugar in the cossettes. A juice content of 21.8 ml. in the normal weight of pulp is assumed, and on this basis the water to be added, in milliliters, is 6.846 times the half-normal weight in grams.

The sugar content found by this method is 0.2 to 0.25 per cent lower than that obtained by the usual hot aqueous digestion. Staněk and Pavlas ascribe this difference to the drying out of the cossettes during mixing and grinding in the usual process. But this conclusion is correct only if it can be shown that the sugar is completely extracted by the substitute method.

ERRORS OF DIGESTION METHODS

Variation in Marc Content and Colloid Water. The flask volumes or amounts of lead water adopted in various countries or districts to correct for the volume of the marc and lead precipitate (see pp. 360–369) are average values, and individual beets may differ appreciably from the average. The error due to this cause should generally be well within 0.1 per cent, but with unripe, wilted, or watery beets it may be considerable, and under these conditions the alcohol-extraction method is preferable, although the reliance formerly placed on that method has proved to be unfounded.

[142] *Listy Cukrovar.*, 57, 281 (1938/39).

Effect of Impurities on Polarization. All methods of simple polarization for the determination of sucrose are based on the assumption that the only optically active constituent is sucrose, and that its rotation is unaltered by accompanying impurities. This assumption is rarely justified in practice. Normal beets contain not only sucrose, but also small quantities of invert sugar, raffinose, asparagine, glutamine, and other optically active substances. The rotation of some of these is considerably affected by lead subacetate. Degener[143] has shown that asparagine, which is slightly levorotatory in aqueous solution, becomes strongly dextrorotatory in the presence of lead subacetate solution. The opposite has been found to be true of glutamine.[144] The combined effect of these influences may be an appreciable plus or minus error, or the correct result may be obtained through mutual compensation. To obviate the error due to the effect of basic lead acetate on the asparagine the French chemists add a drop of glacial acetic acid to the filtered solution from the aqueous digestion before polarizing. Asparagine is dissolved only 1 part in 290 parts of 80 per cent alcohol, and this solubility is diminished by the addition of lead subacetate. The asparagine error is therefore negligible in the methods of alcoholic extraction or digestion. But the effect of lead subacetate on the rotation of sucrose in alcoholic solution may amount to several tenths of 1 per cent.[145]

If the digestion is carried out at high temperature the results are usually higher than with cold digestion. This may be due to more complete extraction of sucrose, but the result is not necessarily more exact because at high temperatures pectin and hemicelluloses are partly decomposed, with the formation of dextrorotatory substances. According to Pellet these substances are completely precipitated by the lead subacetate solution, when this reagent is of proper strength (about 30° Bé.) and used in proper amount (5 to 6 ml. per 26 g. of pulp). To insure complete precipitation of all dextrorotatory gums some authorities advise using 7 or 8 ml. of basic lead solution. Herzfeld,[146] however, has shown that lead subacetate in hot solution forms a levorotatory combination with certain constituents of beet pulp and is opposed to the use of more than 5 ml. of the reagent per 26 g. pulp for hot-water digestion.

The extraction of high polarizing dextrorotatory gums is very liable

[143] *Deut. Zuckerind.*, **22**, 65 (1897).

[144] Eisenschimmel, *Z. Zuckerind. čechoslovak. Rep.*, **51**, 337 (1926/27).

[145] Weisberg, *Sucr. belge*, **1887**, No. 8; Claassen, *Deut. Zuckerind.*, **14**, 1589 (1889); Herles, *Z. Zuckerind. Böhmen*, **14**, 427 (1889/90).

[146] *Z. Ver. deut. Zucker-Ind.*, **59**, 627 (1909).

to occur, even with cold-water digestion, if sugar beets are unripe, frost-bitten, diseased, or otherwise abnormal. Under such circumstances the method of extraction with alcohol, in which the gums are insoluble, should be employed.

The agreement between the aqueous digestion and alcoholic extraction methods upon normal sugar beets is usually very close. As to which of the water-digestion methods is preferable it may be said that the hot-digestion method is considered more reliable for controlling losses in the factory. The quicker cold-digestion methods should be used only if apparatus is available for securing pulp of extreme fineness. These methods are particularly valuable where rapidity rather than highest accuracy is required, as in serial tests for beet selection.

Sugar Determination in Dried Beets and Steffen Cossettes. The extraction and digestion methods described may be applied also to dried beets and to dried cossettes from the Steffen hot-juice process. Both these materials are high in sugar and also in marc content. For this reason the analyses are usually made upon the half-normal weight in a total volume of 200 ml., and the result must be multiplied by 2, if 400-mm. tubes are used. The samples are prepared by grinding in a feed mill, care being taken that no moisture is lost through excessive heating. For cossettes that have taken up moisture and become sticky, Spengler and Zablinsky[147] recommend a modified meat chopper in which the perforated plate has been replaced by rotating shearing knives.

The analytical methods used are essentially the same as described for dried pulp on p. 376, but 13 ml. of lead subacetate solution is added in the alcohol-extraction or the Pellet hot-water-digestion method, and the lead concentration of the lead water employed in the hot Sachs-Le Docte method must be correspondingly increased. Spengler, Paar, and Mück[148] have found that the marc volume of dried beets upon digestion with water is 4.5 ml. per normal weight, and that for Steffen cossettes 9.6 ml. It would therefore be necessary in the Pellet digestion process to use flasks of 202.25 and 204.8 ml., respectively. But it is more convenient to employ 200-ml. flasks, and to reduce the weight of sample from 13 g. to 12.86 and 12.70 g., respectively. Similarly, with the Sachs-Le Docte method, the 177-ml. pipette is retained, and 11.90 g. of dried beets, or 11.74 g. Steffen cossettes, is weighed out. Under these conditions the two methods check very closely with each other.

Although these methods are simple and rapid they cannot be rec-

[147] *Z. Ver. deut. Zucker-Ind.*, 84, 329 (1934).
[148] *Z. Ver. deut. Zucker-Ind.*, 87, 594 (1937).

ommended for accurate work, because the drying process causes inversion of sucrose, caramelization, and other chemical changes in the cossettes. For more exact results it is necessary to determine the sucrose in the extracts by double polarization, or the invert sugar, before and after inversion, by copper reduction methods.

Herles's Method for Determining Sucrose in Sweet Chocolate.[149] This is another example of methods for determining sugar in materials high in insoluble matter. It is a combination of the methods of Scheibler (p. 313) and of Sachs (p. 315). Two flasks of equal volume (100 ml.) are used. One-half normal weight of the chocolate is weighed into each of the two flasks, and in one of them 13 g. of sucrose is added. The chocolate is moistened with methyl alcohol, hot water is added, and the flasks are whirled to dissolve all the sugar. Four milliliters of lead subacetate solution is added to each flask, the volumes are completed to 100 ml., the mixtures well shaken and filtered, and the filtrates polarized in 200-mm. tubes. If the sucrose added had a polarization of 99.8, and P_1 and P_2 are the readings of the filtrates without and with added sugar, respectively, then the corrected reading x of the chocolate solution is calculated by the formula

$$49.9 : (P_2 - P_1) = x : P_1$$

whence

$$x = \frac{49.9 \times P_1}{P_2 - P_1}$$

The percentage of sugar in the sample equals $2x$, since the half-normal weight was used. If $P_1 = 28.2$ and $P_2 = 81.3$, then x is 26.5, and the percentage of sugar 53.0. According to von Fellenberg and Ruffy[150] this method gives low results because the combination of cocoa paste and lead precipitate adsorbs sucrose; they propose an empirical correction formula.

POLARIZATION OF PLANT SUBSTANCES CONTAINING BUT LOW PERCENTAGES OF SUGAR

The methods previously described may be applied with minor modifications to the polarization of plant substances containing but low percentages of sugar. The polarization of exhausted cossettes (" pulp ") and of sugar-cane bagasse may serve as illustrations of the methods.

Polarization of Exhausted Cossettes by the Expression Method. Although the water circulating through the diffusion battery removes

[149] *Z. Zuckerind. čechoslovak. Rep.*, 57, 256 (1932/33).

[150] *Mitt. Lebensm. Hyg.*, 23, 6 (1932).

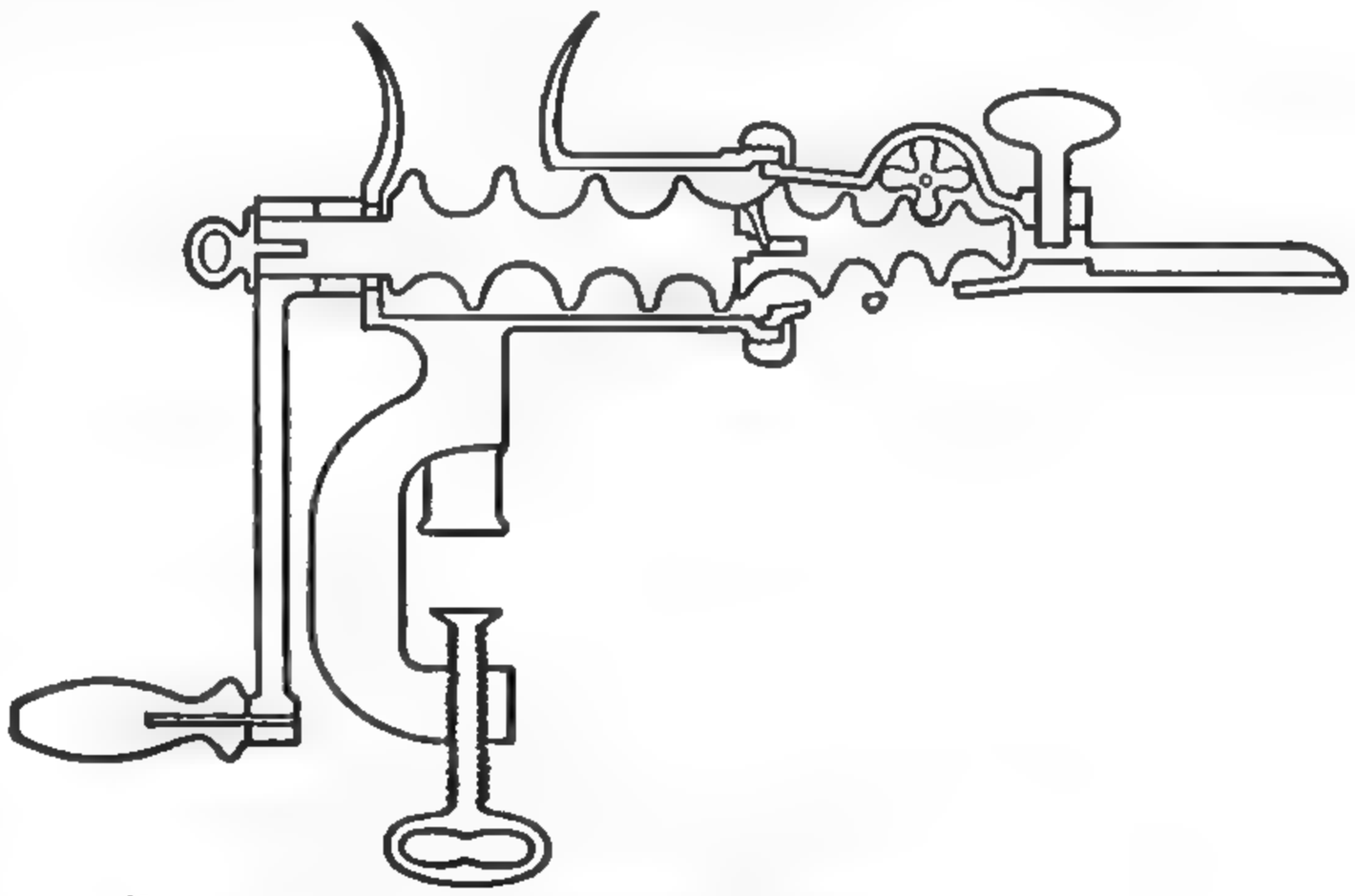
most of the sugar from the beet chips, a small amount of sugar always remains unextracted; this residual sugar occurs for the most part within the uncrushed cells of the beet. It is necessary, therefore, in squeezing out the water from diffusion chips to apply extreme pressure, in order to secure the maximum quantity of residual sugar. A polarization of the expressed diffusion water and a determination of its amount are sufficient for the calculation.

Example. One hundred milliliters of the diffusion water pressed from a sample of spent beet chips was clarified with 2 ml. of lead subacetate solution and the volume completed to 110 ml. The filtered solution gave a polarization of 2.0° V. in a 400-mm. tube. The water content of the chips, upon drying 10 g. at 100° to 110° C. to constant weight, was 90.5 per cent.

The polarization corrected for the dilution is $2.0 \times 1.1 = 2.2°$ V. Calling the specific gravity of the waste diffusion water 1.000 (which can be done without serious error) the polarization of a normal weight would be $(26.00 \times 2.2) \div 100 = 0.572°$ V., or for a 200-mm. tube 0.29° V. The polarization of the spent chips would then be $(90.5 \times 0.29) \div 100 = 0.26$.

The correction for dilution from 100 to 110 ml. may be dispensed with, and the calculation simplified, by adding dry lead subacetate to the expressed diffusion water, mixing, filtering, and polarizing.

The expression method has been criticized by Lippmann, Rümpler, Wohryzek, Linsbauer, and others, principally because the expressed solution does not represent the whole residual juice in the cossettes (see p. 349), and the results are generally too low. The method is not officially recognized in Germany but is still widely used because it is simple and rapid, and the error is usually not significant. In Czechoslovakia a special apparatus,[151] the "Kosmos" press, Fig. 197, is employed to express the juice. The first 50 ml. of liquid is discarded; the next 100 ml. is collected and clarified with 0.75 g. of a ground mixture of



(Reproduced from Z. Zuckerind. Techoslovak. Rep., 55, 175.)

FIG. 197. "Kosmos" press.

[151] *Z. Zuckerind. čechoslovak. Rep.*, 55, 175 (1930/31); 59, 41 (1934/35).

1 part finely powdered caustic lime and 25 parts of dry neutral lead acetate. The filtrate is polarized in a 400-ml. tube, and the reading multiplied by 0.13 to obtain the polarization of the expressed juice. The results of this method check closely with those obtained by the aqueous digestion method.

Polarization of Exhausted Cossettes by Aqueous Digestion.[152] The double-normal weight of pulp, 52 g., is digested according to Pellet's hot-water procedure (p. 363) in a 202.0-ml. flask, 1 to 2 ml. of lead subacetate solution being used for clarification. The hot Sachs-Le Docte method (p. 365) may also be employed, with 60 (i.e. $26 \times 177 \div 77$) g. of pulp and 177 ml. of lead water (25 ml. subacetate solution and 1 liter of water). In either case the filtrate is polarized in a 400-ml. tube and the reading divided by 2 to obtain the polarization of the exhausted cossettes.

Polarization of Dried Pulp by the Aqueous Digestion Method. The sample is prepared by grinding in a feed mill. Since Spengler, Paar, and Mück[153] have found a volume of 15 ml. for the hydrated marc obtained upon digestion of the normal weight of dried pulp with water, a flask of 207.5-ml. capacity would have to be used for the half-normal weight, in the Pellet digestion method. It is more convenient to employ a 200-ml. flask and to reduce the weight of sample to 12.53 g. Water, and 6 to 7 ml. of lead subacetate solution, are added, and the analysis is carried out by the Pellet hot-water-digestion procedure (p. 363). If the Sachs-Le Docte method is preferred, 11.57 g. of sample is treated with 177 ml. of lead water, prepared by mixing 150 ml. of lead subacetate solution with 1 liter of water; the determination is carried out as shown on p. 365. The reading obtained by either method in a 400-ml. tube is multiplied by 2 to find the sugar content of the pulp.

Alcohol Digestion-Extraction Method of Rössing.[154] Dried pulp has frequently undergone a change in composition through formation of water-soluble optically active gums at the high temperature of drying. The aqueous digestion method may then give a polarization different from the true sucrose content. In such cases it is recommended to use the alcoholic digestion and extraction method.

Rössing has modified Herzfeld's original method[155] as follows: The half-normal weight of sample is treated in the sugar weighing dish

[152] Frühling's "Anleitung," 9th ed., p. 272, 1919; 10th ed., by Spengler, p. 197, 1932.

[153] *Z. Ver. deut. Zucker-Ind.*, **87**, 594 (1937).

[154] Frühling's "Anleitung," 9th ed., p. 274, 1919; 10th ed. by Spengler, p. 199, 1932.

[155] *Z. Ver. deut. Zucker-Ind.*, **59**, 638 (1909).

with 30 ml. of hot water and 6 ml. of lead subacetate solution for 15 minutes with frequent stirring, to hydrate the marc. The mixture is transferred with about 30 ml. of hot water and then with 90 per cent alcohol to the extractor (see p. 353) in the bottom of which is placed a wad of glass wool covered with wire gauze. The pulp must exactly fill the space up to the upper bend of the siphon tube, to assure rapid extraction. The space should be about 100 mm. high and 38 mm. in diameter. The extract is collected in a 200-ml. volumetric extraction flask of the Kohlrausch type or of the form shown in Fig. 188. Sufficient 96 per cent alcohol is placed in the flask so that after siphoning the liquid fills about two-thirds of the flask. Complete extraction requires about 2 to 3 hours and is checked by the α-naphthol test. After cooling the flask is filled to the mark and the solution is filtered and polarized. The reading in a 400-ml. tube, multiplied by 2, gives the sugar content of the dried pulp.

Neither this method nor the water-digestion method gives correct results if the pulp has been dried at a temperature high enough to cause inversion and caramelization. In such cases the sucrose and invert sugar must be determined in the extract.

Polarization of Sugar-Cane Bagasse by Hot-Water Extraction. To prepare sugar-cane bagasse for analysis, it should be finely chopped in a Warmoth disintegrator, a meat-chopping machine, or similar apparatus which reduces it to pieces not over 0.5 cm. in any dimension. The moisture loss should be determined by weighing the bagasse before and after chopping.

The hot-water extraction method of Zamaron (p. 357) may be employed upon bagasse in the same manner as described for sugar cane. Owing, however, to the much larger amount of cellular matter in bagasse only 50 g. is taken for extraction. The extract is made up to 1500 ml. and polarized in a 400-ml. tube. The reading multiplied by 3.9 gives the polarization of the bagasse.

Extraction waters of very low sugar content are sometimes concentrated before polarization. Five hundred milliliters of the neutralized solution is evaporated to somewhat less than the desired volume, and then made up to 100 ml. or 250 ml. for polarization. The saccharimeter reading is divided by 5 or 2 to obtain the polarization of the extract.

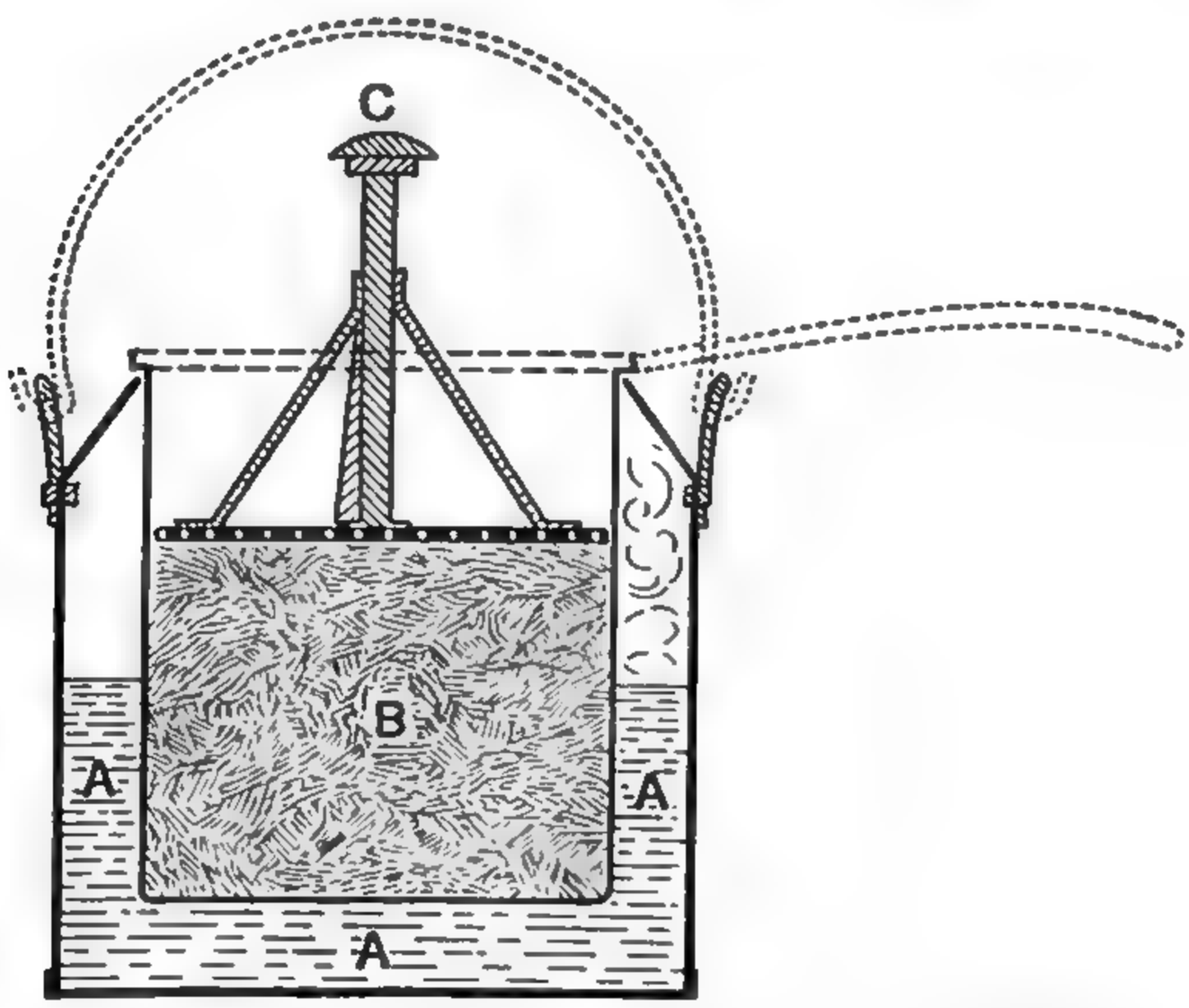
The extraction method is rarely used in practice upon bagasse, some form of digestion procedure being preferred. In this case it is necessary to know the percentage of fiber. This may be determined by the methods described for determining fiber in sugar cane (p. 348), but variations in the fiber content of the bagasse have

such a small effect on the result that it is customary to assume an average figure for the fiber content.

Of the numerous digestion methods in use, three will be given as examples.

Hot-Water Digestion Method of Norris. This is the official method of the Association of Hawaiian Sugar Technologists.[156]

The Norris digester, Fig. 198, consists of: (*A*) an outside cylindrical vessel for boiling water, 15 cm. high and 13 cm. in diameter, crimped in at the top so that the inside vessel fits in snugly; (*B*) an inside cylindrical vessel for digesting the sample, 11 cm. high and 11 cm. in diameter, with a straight handle and a rolled edge on top, upon which it rests in the outside vessel;



(Reproduced with permission from "Methods of Chemical Control," Assoc. Hawaiian Sugar Tech., 2nd ed., p. 18.)

FIG. 198. Norris bagasse digester.

(*C*) a tamp made of a disk of heavy metal with numerous holes and a rigid handle for pressing down on the bagasse. The latter should fit rather tightly into the inside vessel. The tamp serves as a cover during digestion by resting on lugs at the top of *B*. The digesters should be substantially made of copper. They may be heated in individual water baths or more conveniently in a large bath accommodating several digesters. A 500-ml. metal container for measuring the hot water should be provided.

Weigh a sample equivalent to 100 g. of the original bagasse into the tared digester. Add 500 ml. of hot water containing 5 ml. of a solution of sodium carbonate of 12.5 Brix, press the bagasse down into the solution, and place the cup in the outside vessel containing boiling water. Digest for 1 hour, mixing the solution with the bagasse every 15 minutes by pressing down

[156] "Methods of Chemical Control," 2nd ed., pp. 17 and 38, 1931.

with the tamp, and using the latter for a cover for the digestion cup between times. Do not add any more water. Allow the mixture to cool a little and weigh (W). Press out as much of the solution as possible with the tamp, cool to room temperature, clarify by adding 0.3 to 0.5 g. dry lead subacetate mix, filter, and polarize in a 400-mm. tube.

The polarization is reduced to the normal-weight basis by multiplying by 26 and dividing the product by 2×100. The polarization of the bagasse is then found by the formula

$$\frac{26\,P}{2 \times 100} \times \frac{(W - F)}{100} = \frac{P(W - F)}{7.69 \times 100}$$

where F is the percentage of fiber in the bagasse, and $(W - F)$ the weight of solution corresponding to 100 g. of bagasse.

Instead of clarifying with dry lead, 99 ml. of the solution may be completed to 100 ml. by the addition of lead subacetate solution, 7.6 being substituted for 7.69 in the formula.

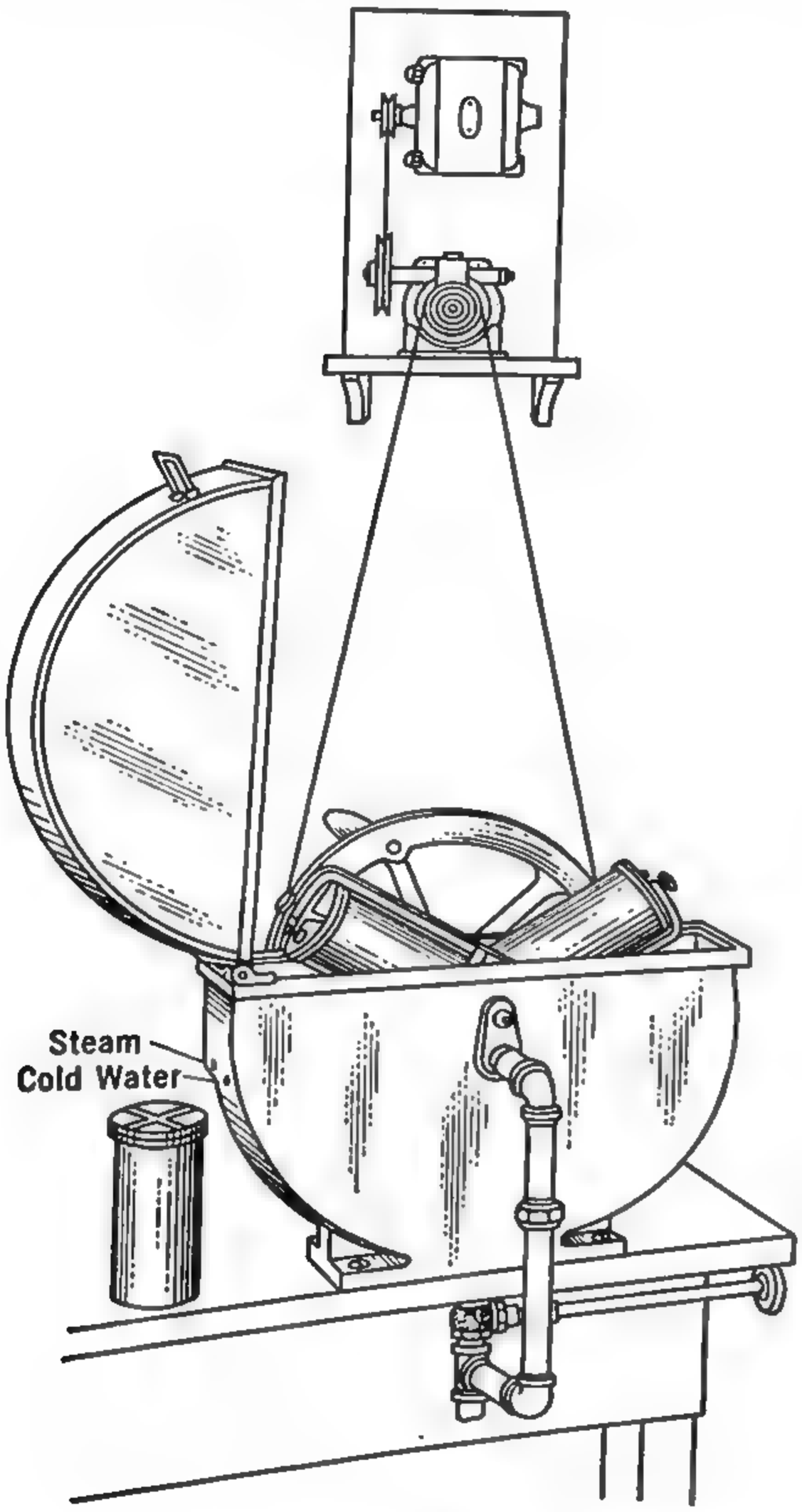
For polariscope readings up to 2.0 a fiber content of 60 per cent is assumed, for readings above 2.0 and up to 4.0 a fiber content of 55 per cent, and for readings above 4.0 a fiber content of 50 per cent. In other countries 50 or 48 per cent fiber is used for F.

In many places a simpler digester is employed, consisting either of a cylindrical vessel (copper or brass) about 4 inches in diameter and 6 inches high, with a clamped cover, or a bottle-shaped digester, 8 inches high and 4 inches in diameter, reduced at the top to 2 inches and closed with a rubber stopper. In either apparatus a brass tube is inserted in the cover to act as a condenser, and a brass rod with a disk at the lower end passes through the brass tube, to be used for stirring. The bagasse and water are placed in the digester, and this is immersed in a boiling-water bath.

Spencer Rotary Digester.[157] In order to provide continuous agitation during the digestion and promote the extraction of the sugar, Spencer devised the apparatus shown in Fig. 199. It consists of a cylindrical casing, 24 in. in diameter, with inlets for steam and cold water, and a drain pipe. The upper part of the casing is hinged to serve as a cover. Inside of the casing are three detachable aluminum cylinders, 8 inches long by 4.5 inches in diameter, mounted symmetrically on the center shaft, and provided with tight-fitting covers having air vents. These digesters are rotated at 5 revolutions per minute by means of a pulley. Each cylinder is filled with 100 g. of bagasse, 1 liter of very hot water is added, and the cover put on. The steam is turned on while the drain pipe is open, and the cylinders

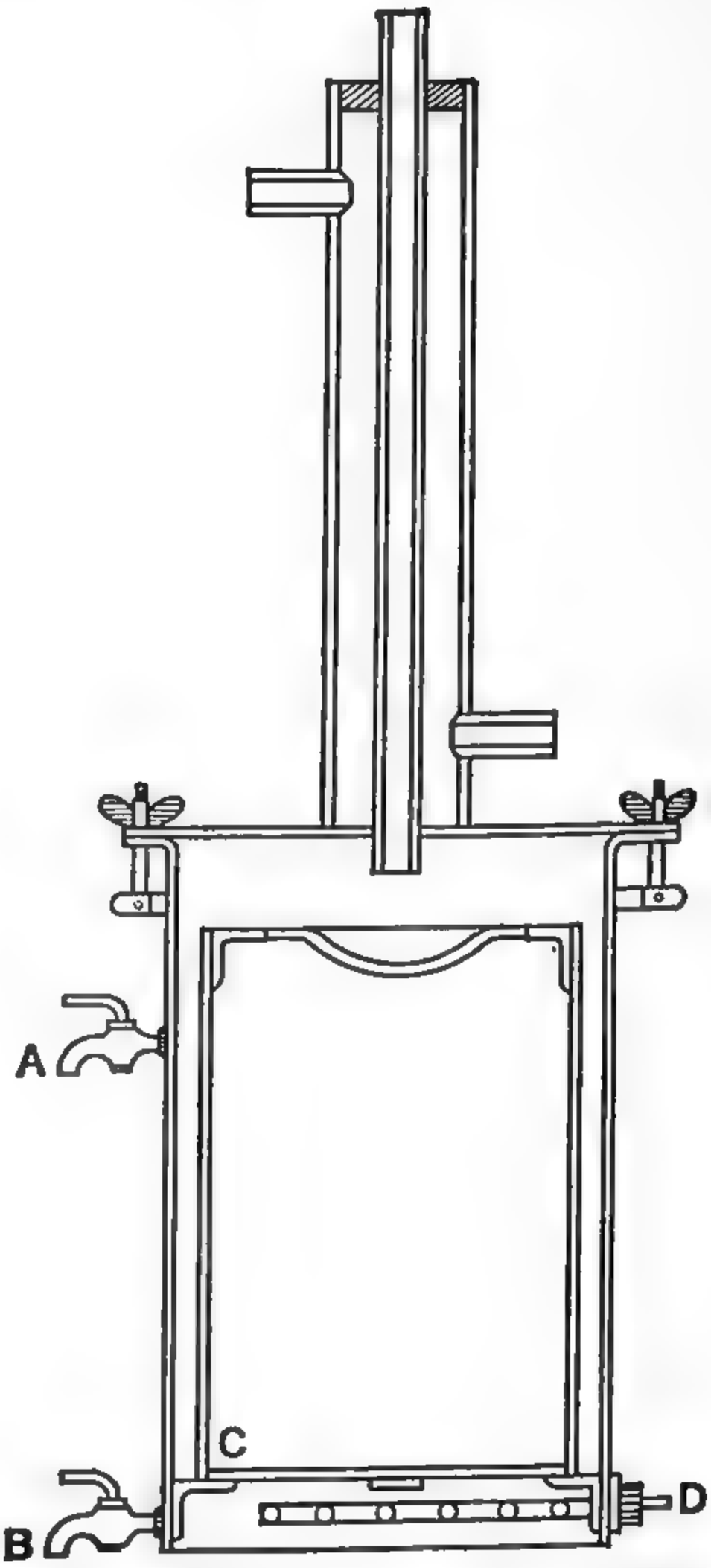
[157] *Ind. Eng. Chem.*, 13, 640 (1921).

are revolved for one hour. Then the steam is shut off, the drain closed, and cold water is admitted, the rotation being continued until the samples have cooled. The cylinders are removed, wiped dry, and



(Courtesy of Eimer and Amend.)

FIG. 199. Spencer's rotary bagasse digester.



(Reproduced with permission from "Methods of Chemical Control," Sugar Tech. Assoc. India, p. 101.)

FIG. 200. Deerr's digester for large samples of bagasse.

weighed. They are then opened, the solution is strained from the bagasse, and the analysis is completed as described for the Norris method.

Method of Deerr.[158] In this process much larger quantities of bagasse and water are employed, and the mixture is actually boiled.

[158] *Intern. Sugar J.*, 17, 213 (1915); "Methods of Chemical Control, Sugar Technologists' Assn. of India," p. 100, 1936.

The bagasse need not be finely chopped, but can be used as it comes from the mill. The principle is similar to that of the Sachs-Le Docte method, with a definite ratio between bagasse and water, so that the final solution is one-half normal. The digestion vessel, Fig. 200, is made in two sizes, for approximately 500 g. or 1000 g. of bagasse. The exact quantity of water is fixed by the level of the upper cock on the side of the vessel. A steam coil in the bottom is used for heating, and a reflux condenser is attached to the cover which is tightly bolted to the upper rim. The capacity of the digester is determined by filling it with water until it overflows through the upper cock. The quantity of bagasse x to furnish a half-normal solution is found from the equation:

$$\frac{(W + ax)}{x} = \frac{100}{13} = 7.69; \qquad x = \frac{W}{7.69 - a}$$

where W is the water capacity of the vessel in milliliters, and a the percentage of moisture in the bagasse divided by 100. Supposing that the digestion vessel holds 7215 ml. of water, and the average moisture content of the bagasse is 46 per cent, the weight of bagasse to be taken is 7215/ (7.69 — 0.46) = 998 g.

The digester is filled with water, the excess being allowed to overflow through the upper cock which is then closed. The calculated quantity of bagasse is placed in the perforated metal basket which is then dropped into the digester. The cover is put in place, the cooling water for the condenser turned on, and the contents of the vessel are boiled for 1 hour. The extract is then withdrawn through the lower cock and cooled to room temperature; a portion of it is clarified with dry lead subacetate, mixed, filtered, and the filtrate polarized in a 400-mm. tube. The reading gives directly the polarization of the bagasse.

The apparatus and method of Khainovsky,[159] used in Java, is similar to that of Deerr, but a definite ratio of 1 kg. bagasse to 10 liters of water is employed, and the polarization is reduced to the normal-weight basis by calculation.

Errors of the Bagasse Digestion Methods. In the polarization of bagasse the same difficulties arise as in that of beets or cossettes. The sugar must be completely extracted, but inversion of sucrose, and the solution of pentosans, pectins, and other impurities affecting the polarization must be avoided. Other conditions being equal, actual boiling, as practiced in Deerr's method, is likely to give higher results

[159] *Arch. Suikerind.*, 35, III, 107 (1927); 36, III, 843 (1928); 38, III, 943 (1930).

than heating in a water bath. It is generally conceded that digestion for 60 minutes at about 100° C. extracts all the sucrose, provided that sufficient water is used and that there is perfect admixture.

Dymond[160] contends that heating at 50° C. for 2½ to 3 hours gives a truer, though lower, result, but Khainovsky[161] has shown that the sugar cannot be extracted from unbroken cells until after the protoplasm has been killed by boiling.

The invert sugar in bagasse is negligible, and inversion of sucrose is prevented by keeping the solution slightly alkaline. Prinsen Geerligs[162] showed that prolonged boiling extracts a dextrorotatory gum from bagasse, but Norris[163] was unable to confirm this for Hawaiian bagasse. Moreover, de Haan[164] found that the gum is precipitated by lead subacetate, and does not interfere. Hedley and Hayes[165] were able to identify xylose in the extract obtained by prolonged boiling of bagasse with water alone, after the sucrose had been completely extracted with cold water. But this pentose was probably formed from xylan by hydrolysis in the slightly acid solution.

Another error arises from the colloid water in the bagasse when the polarization is calculated on the basis of the dry fiber content, and from the soluble solids when it is based on the water content of the bagasse instead of the juice content. But these errors are usually within the limits of accuracy of polarimetric readings.

POLARIZATION OF SUBSTANCES CONTAINING INSOLUBLE MINERAL MATTER

The polarization of substances containing insoluble mineral matter can in general be carried out by the methods of extraction or digestion previously described. Certain classes of products, however, such as carbonatation filter-press cake may contain sugar in the form of insoluble saccharates, and for them special methods of treatment are required. As examples of methods to be employed several processes for the polarization of filter-press cake will be described.

Polarization of Filter-Press Cake Free from Saccharate. If saccharate-free press cake is triturated with a known quantity of water and the filtered extract polarized, the polarization of the cake may be

160 *Proc. 5th Annual Congr. S. African Sugar Tech. Assoc.*, p. 40 (1931).
161 *Arch. Suikerind.*, 35, II, 811 (1927).
162 *Arch. Suikerind.*, 16, 171 (1908).
163 *Expt. Sta. Hawaiian Sugar Planters' Assoc., Bull.* 32, 1910.
164 *Arch. Suikerind.*, 18, 118 (1910).
165 *Proc. 8th Annual Congr. S. African Sugar Tech. Assoc.*, p. 4 (1934).

calculated very closely, provided that its moisture content has been determined.

Example. Fifty grams of press cake was ground in a mortar with 200 ml. of water. The solution (which should not be alkaline) was then clarified with a little dry lead subacetate and polarized in a 400-mm. tube. A reading of 5.2° V. was obtained. The moisture content of the cake, determined by drying 10 g. in a hot-water bath to constant weight, was 45.6 per cent. It is desired to know the polarization of the cake.

The weight of water in the 50 g. of cake is $50 \times 0.456 = 22.8$ g. The total volume of liquid (disregarding the slight increase in volume through solution of sugar) is then $200 + 22.8 = 222.8$ ml. The polarization of the solution reduced to a normal weight of 26 g. to 100 ml. (calling the specific gravity 1.000, which may be done without serious error) is $(5.2 \times 26) \div 100 = 1.35°$ V., which for a 200-mm. tube is 0.68° V., or 0.68 g. of sucrose in 100 ml. of solution. This corrected to 222.8 ml. $= 0.68 \times 2.228 = 1.52$, the grams of sucrose in 50 g. of cake; $1.52 \times 2 = 3.04$, the polarization or percentage of sucrose in the cake, if no other optically active substances are present.

The above method of calculation is sufficiently exact for substances of low polarization. When the polarization is high, however, neglect of the increase in volume through solution of sugar and of the change in specific gravity introduces a considerable error. In such cases the polarization should be determined by some method of extraction.

In sugar-house practice the determination of moisture in the press cake is usually dispensed with, it being assumed that the volume of insoluble matter in 26 g. of cake is 4 ml. The normal weight of cake is then made up to 104 ml.; or, if a 100-ml. flask is used, 25 g. of cake, when triturated, clarified with lead solution, and the liquid made up to volume, will give the polarization (104 : 26 : : 100 : 25). In practice 50 g. of cake is generally weighed out and the volume made up to 200 ml.

In the previous example if the 50 g. of cake had been made up with water to 200 ml., there would be 192.3 ml. of solution (allowing 4 ml. for volume of insoluble matter in 26 g.). The polarization for 222.8 ml. of solution was 5.2° V., therefore 192.3 : 5.2 : : 222.8 : 6.02, the calculated polarization of the cake for a 400-mm. tube. This for a 200-mm. tube would be 3.01, which is only 0.03° V. lower than the result previously found.

When the carbonatation process is employed, as in the beet-sugar industry and to some extent in the cane-sugar industry, the "free" sugar, i.e., that not combined with lime, is also determined as just described. But more generally 53 g. of press cake is weighed out, and 177 ml. of lead water, containing 3 ml. of lead subacetate solution,

is added from a Sachs-Le Docte pipette to extract the sugar. The press cake contains about 50 per cent of water, and therefore the double-normal weight requires 174 ml. to complete the volume to 200 ml. The weight of press cake corresponding to 177 ml. is $52 \times 177 \div 174 = 52.9$, or rounded off 53 g. In Germany and Czechoslovakia 177 ml. of pure water is added instead of lead water because the lead subacetate is likely to precipitate sucrose from the alkaline solution.

Polarization of Filter-Press Cake Containing Saccharate. When filter-press cake contains insoluble saccharates, the sugar must be liberated from combination before the solution to be polarized is made up to volume. Several methods have been followed for accomplishing this result.

Decomposition of Saccharate by Means of Acetic Acid. The 50 g. of press cake, after transferring with water to a 200-ml. flask, is heated to boiling, and acetic acid added drop by drop until all free alkali is neutralized. The solution is then cooled, clarified, made up to volume, filtered, and polarized as previously described.

Decomposition of Saccharate by Means of Carbon Dioxide. The method is practically the same as that just described, except that a stream of carbon dioxide led into the solution is used for decomposing the saccharate, instead of acetic acid.

The frothing, caused by evolution of carbon dioxide, is the principal objection against the acetic acid method, and the decomposition by means of carbon dioxide usually requires considerable time. Methods have been devised, therefore, to decompose insoluble saccharates in other ways. One of the most common of such methods is the following:

Decomposition of Saccharate by Means of Ammonium Nitrate. The saccharates of calcium are quickly decomposed by ammonium nitrate with the formation of free sugar, calcium nitrate, and ammonia. The reaction for monocalcium saccharate is

$$\underset{\text{Saccharate}}{C_{12}H_{22}O_{11}CaO} + 2\,NH_4NO_3 + H_2O \rightarrow \underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + Ca(NO_3)_2 + 2\,NH_4OH$$

In carrying out the process 50 g. of press cake is ground up with 15 g. of ammonium nitrate and 100 ml. of cold distilled water. The mixture is then washed into a 200-ml. flask, clarified with a little lead subacetate solution, made up to volume, and polarized in the usual way.

An objection against the ammonium nitrate method is the liberation of free ammonia, which in the presence of the lead-clarifying agent may precipitate a part of the sucrose as lead saccharate. For this reason the German official method omits the use of lead subacetate; 53 g. of press cake is triturated in a mortar with 177 ml. of 10 per

cent ammonium nitrate solution, added from a Sachs-Le Docte pipette or a measuring cylinder.

The free ammonia sometimes causes a darkening of the solution; contact with the brass fittings of polariscope tubes may color the ammoniacal solution blue. Care should be exercised, therefore, to prevent contact of the solution with copper or brass during the analysis.

According to Spengler and Brendel[166] the acetic acid or ammonium nitrate method sometimes gives low results, because in the presence of certain colloids the saccharate is not completely decomposed at room temperature. In such cases the wet press cake must first be heated in a closed vessel to 85–90° C. for 1 hour before the ammonium nitrate solution is added.

Decomposition of Saccharate by Means of Zinc Nitrate. In order to eliminate the formation of free alkali Staněk[167] has proposed the employment of zinc nitrate for decomposing the saccharate. The reaction proceeds as follows:

$$\underset{\text{Monosaccharate}}{C_{12}H_{22}O_{11}CaO} + \underset{\text{Zinc nitrate}}{Zn(NO_3)_2} + H_2O \rightarrow \underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + \underset{\text{Calcium nitrate}}{Ca(NO_3)_2} + \underset{\text{Zinc hydroxide}}{Zn(OH)_2}$$

The precipitated zinc hydroxide is removed with the insoluble mineral matter of the cake, and a perfectly neutral filtrate is obtained.

In carrying out the process, 53 g. of saccharate cake is weighed into a metal capsule with cover and rubber envelope or a wide-mouth bottle with cork, 177 ml. of a 10 per cent solution of commercial zinc nitrate is added gradually, a piece of metal chain is put in to break up the lumps, and the covered capsule or bottle is vigorously shaken until the cake is completely disintegrated. The mixture is filtered and the filtrate polarized in a 200-mm. tube.

According to Staněk and Nemes[168] the zinc nitrate may be replaced by the much cheaper zinc chloride, a solution of which is prepared in the laboratory by treating 500 g. of metallic zinc with 1 kg. of dilute hydrochloric acid (1 plus 1). When the reaction is complete the zinc chloride solution is decanted, a few small pieces of limestone are added to neutralize the free acid, and the solution is diluted to about 10° Brix. This solution is then used instead of the 10 per cent zinc nitrate solution.

The methods which have been described for polarizing products of the cane- and beet-sugar industry may be applied equally well to the polarization of other sucrose-containing substances, such as maple and sorghum products, jellies, preserves, and confections. The same

[166] *Z. Ver. deut. Zucker-Ind.*, **79**, 61 (1929); **80**, 69 (1930).
[167] *Z. Zuckerind. Böhmen*, **34**, 161 (1909/10).
[168] *Z. Zuckerind. čechoslovak. Rep.*, **55**, 451 (1930/31).

methods may also be applied to the polarization of substances which contain other sugars than sucrose, the only change necessary being in the constant for the normal weight. As an example of the application of saccharimetric methods to other sugars besides sucrose, the determination of milk sugar in milk is selected.

SACCHARIMETRIC DETERMINATION OF LACTOSE

Polarization of Milk.[169] The normal weight of lactose for a saccharimeter with the Ventzke sugar scale may be taken as 32.9 g. (see p. 298). Owing to the low percentage of lactose in milk (2 to 8 per cent) it is best to employ double the normal weight, and, as it is more convenient to measure the milk, tables have been prepared which give the volumes of milk corresponding to multiples of the normal weights for different saccharimeters. Table LXI gives the volumes of milk for 65.8 g. which correspond to different specific gravities.

TABLE LXI

VOLUMES OF MILK CORRESPONDING TO A LACTOSE DOUBLE-NORMAL WEIGHT

Specific Gravity of Milk	Volume of Milk for a Lactose Double-Normal Weight (Ventzke scale)	Specific Gravity of Milk	Volume of Milk for a Lactose Double-Normal Weight (Ventzke scale)
	ml.		ml.
1.024	64.25	1.031	63.80
1.025	64.20	1.032	63.75
1.026	64.15	1.033	63.70
1.027	64.05	1.034	63.65
1.028	64.00	1.035	63.55
1.029	63.95	1.036	63.50
1.030	63.90	.....	.....

For ordinary purposes a pipette graduated to deliver 64 ml. is sufficiently exact.

Acid Nitrate of Mercury Solution. In clarifying milk for polarization acid nitrate of mercury is generally used. The reagent is prepared as follows: Dissolve metallic mercury in twice its weight of nitric acid of 1.42 sp. gr., and dilute with an equal volume of water.

Mercuric Iodide Solution. Mercuric iodide solution may also be used for clarification. The reagent is prepared by adding 33.2 g. of

[169] "Methods of Analysis, A. O. A. C.," 4th ed., p. 266, 1935. Certain changes in clarification proposed by Garrison and described on p. 387 are made in the 5th edition of "Methods of Analysis, A. O. A. C.," p. 271, 1940.

potassium iodide to a solution of 13.5 g. mercuric chloride in 200 ml. of glacial acetic acid and 640 ml. of water.

In carrying out the process, the volume of milk corresponding to the lactose double normal weight is measured into a 102.6-ml. flask. For clarification either 1 ml. of the acid mercuric nitrate, or 30 ml. of the mercuric iodide solution may be used (an excess of either reagent does no harm). The liquid is shaken and then made up to a volume of 102.6 ml., the extra 2.6 ml. being the estimated volume of the precipitated casein, albumin, and fat. After shaking frequently for at least 15 minutes, the liquid is filtered and polarized in a 400-mm. tube; the scale reading divided by 4 gives the approximate percentage of lactose in the milk.

According to Garrison[170] the mercuric nitrate or iodide reagents do not give satisfactory clarification for abnormal milks, such as colostrum or mastitis milk but good results are obtained with Somogyi's zinc hydroxide reagent.[171] A solution of 15 g. zinc sulfate ($ZnSO_4 \cdot 7\,H_2O$) in 100 ml. total volume, and a 0.75 *N* sodium hydroxide solution, are prepared. The solutions are standardized against each other so that 10 ml. of the zinc sulfate solution, diluted with 60 ml. of water, requires from 10.9 to 11.1 ml. of the sodium hydroxide solution, to produce a permanent pink color with phenolphthalein indicator. To clarify the milk, 17.5 ml. of the zinc sulfate solution is added, the mixture is well shaken, and then 17.5 ml. of the sodium hydroxide solution is run in with continuous shaking. The volume is completed to the mark, and the polarization finished in the usual way. Normal milks require a smaller quantity of the clarifying agents than abnormal milks.

Garrison also found that the 1 ml. of mercuric nitrate solution specified in the method of the Association of Official Agricultural Chemists is insufficient for complete removal of the protein, and that 2 or even 3 ml. should be used. The protein removal is facilitated by adding 25 ml. of a 5 per cent solution of phosphotungstic acid before the addition of the mercuric nitrate.

Wiley and Ewell's[172] *Double-Dilution Method.* The volume of precipitate in the preceding method varies according to the content of protein and fat so that a fixed estimate is not always accurate. For more exact purposes of analysis the double-dilution method of Wiley and Ewell may be used. The general principle of double dilution, due to Scheibler, has been considered on p. 313.

Two separate double lactose-normal-weight portions of milk are in-

[170] *J. Assoc. Official Agr. Chem.*, 18, 408 (1935).
[171] *J. Biol. Chem.*, 66, 655 (1930).
[172] *Analyst*, 21, 182 (1896).

troduced into a 100- and 200-ml. flask, respectively. The same volume of clarifying agent is then added to each flask and the volume completed to the mark. The solutions are shaken, filtered, and read in a 400-mm. tube. The reading of the 100-ml. solution subtracted from 4 times the reading of the 200-ml. solution gives the reading corrected for volume of precipitate, and this reading divided by 4 gives the percentage of lactose in the milk.

Example. The saccharimeter readings (400-mm. tube) of a milk analyzed by the above method were 20.00 for the 100-ml. flask and 9.80 for the 200-ml. flask.

The reading corrected for volume of precipitate is then $(4 \times 9.80) - 20.00 = 19.20$, and the percentage of lactose is $19.20 \div 4 = 4.80$.

The volume of precipitate according to the above observations would be

$$\frac{100\ (20.0 - 19.2)}{20} = 4 \text{ ml.}$$ (see p. 314)

Leffmann and Beam's Method. When the percentages of fat and protein are known in a milk, the volume of precipitate formed during clarification can be calculated according to Leffmann and Beam[173] by the following method.

Calling the specific gravity of milk fat 0.93 the volume of precipitated fat is found by multiplying the grams of fat in the weight of sample by $1/0.93 = 1.075$. In the same way the volume of the precipitated protein-mercury compound is found by multiplying the grams of protein in the weight of sample by $1/1.25 = 0.8$. The sum of the volumes of fat and protein is the volume in milliliters of the precipitate.

Garrison[174] found the volume of the combined fat and protein precipitate for 65.8 g. of milk from 34 cows to vary from 5.01 to 8.51 ml., which is much higher than the 2.6 ml. assumed in the method of the Association of Official Agricultural Chemists. For this reason this method gave results from 0.05 to 0.30 per cent higher than when the proper individual correction was applied. The results of the double-dilution method were in most cases still lower than those corrected individually, but in a few instances they were a little higher. This point requires further investigation.

For the polarization of evaporated or condensed milks the single lactose-normal-weight of substance is taken, or the product is first diluted with water, the determination made by the method used for milk, and the result corrected for dilution. The method of analysis in other respects is the same as described for ordinary milk.

[173] "Analysis of Milk and Milk Products," p. 39, 1896.

[174] *J. Assoc. Official Agr. Chem.*, **22**, 489 (1939).

The determination of lactose in milk by the saccharimeter is not considered upon the whole to be as accurate as by the gravimetric method of copper reduction. A considerable variation is frequently found in the determinations by the two methods. In ten comparative determinations of lactose in condensed milk by different collaborators of the Association of Official Agricultural Chemists[175] an average variation of $\pm$ 0.30 was found between the results by the optical and by the gravimetric method, the differences ranging from 0.03 to 0.90. In a series of comparative determinations by Patrick and Boyle[176] upon unsweetened condensed milks, the following results were obtained:

Sample	Lactose	
	By Polariscope, Clarification with Acid $Hg(NO_3)_2$	By Copper Reduction, Soxhlet's Method
1	10.07	10.04
2	10.19	10.51
3	10.57	10.69
4	9.97	10.15
5	8.71	9.20
6	9.00	9.37

The correction for volume of mercury precipitate in the above samples was made by the method of Leffmann and Beam. It is seen that there is an average difference of about 0.25 between the two methods.

The cause of the occasional wide deviations between the results of the optical and gravimetric methods for determining lactose has been variously explained. The difference has been attributed by some to the presence of foreign optically active substances, such as unprecipitated proteids, organic acids, "animal gum," etc., but this has not been conclusively established. Differences due to variation in volume of precipitated fat and proteids are of course greater in condensed or evaporated milks.

Polarization of Milk Sugar. The optical method for determining lactose is easily applied to the analysis of commercial milk sugar, when other optically active compounds are absent. The lactose-normal-weight of sugar is made up to 100 ml. with the addition of a little alumina cream; with dark-colored products containing milk sugar the solution of substance must be clarified, following the same methods and precautions as in the polarization of raw cane sugars.

[175] *Proc. A. O. A. C.*, 1906, 1907, *Bulls.* 105 and 116, U. S. Bur. Chem.
[176] *Bull.* 105, U. S. Bur. Chem., p. 109.

In polarizing milk sugar the saccharimeter reading must not be taken until mutarotation has disappeared; the solution of sugar is either allowed to remain in the tube until a constant reading is obtained or the mutarotation is destroyed by adding a few milliliters of 0.1 *N* sodium carbonate solution at the time of making up to volume.

The methods of simple polarization described in the present chapter may obviously be applied to the polarization of products containing glucose, maltose, and other sugars. But in practical work it is found that such sugars generally occur in mixtures with other carbohydrates, and the methods for their determination are accordingly given elsewhere.

INFLUENCE OF TEMPERATURE UPON SACCHARIMETRIC OBSERVATIONS[177]

Before concluding this chapter upon methods of simple polarization, the influence of changes in temperature upon the accuracy of saccharimetric observations should be considered.

It has been shown (p. 271) that with an increase in temperature the specific rotation of sucrose undergoes a decrease and the rotatory power of the quartz compensation an increase, the combined effect of all influences producing a decrease in the saccharimeter reading of a normal weight of pure sucrose of 0.03° V. for 1° C. increase in temperature, and that for temperatures between 20° and 30° C. the general equation $V^{20°} = V^t\{1 + 0.0003\ (t - 20)\}$ may be used for changing the Ventzke reading (V^t) of pure sucrose at any temperature t to the reading at 20°.

Saccharimeter Temperature Corrections. A temperature correction similar to the above was employed by the United States Treasury Department in 1897, in its polarization of sugars assessed for duty. The right of the Treasury Department to make such corrections in the observed saccharimeter readings was contested in the courts by several importers of sugar, who founded their case largely upon the claim that the rotation of pure sucrose is not appreciably affected by changes in temperature. The chemists representing the government were successful, however, in showing that the specific rotation of sucrose is thus

[177] For a full discussion of this question with bibliographic references see paper by Browne, "The Use of Temperature Corrections in the Polarization of Raw Sugars and Other Products upon Quartz Wedge Saccharimeters," read before Section V, Seventh International Congress of Applied Chemistry, London, 1909, also in *J. Ind. Eng. Chem.*, 1, 567, and *Z. Ver. deut. Zucker-Ind.*, 59, 404.

affected, and after a final appeal to the United States Supreme Court the case of the importers was dismissed for want of jurisdiction.[178]

The decision of the courts, which apparently justified the use of temperature corrections established for pure sucrose in correcting the polarization of all grades of raw sugars, has unfortunately seemed to many chemists sufficient authorization to use such corrections indiscriminately in the polarization of any and every kind of sugar-containing material. Since the saccharimetric reading of a raw sugar or other impure product is simply an expression of the sum of the optical activities of the various constituents, sucrose, glucose, fructose, organic acids, gums, etc., it is evident that a system of temperature corrections which will give the saccharimeter reading that would be obtained at 20° C., must correct for the variations produced by temperature in the specific rotation of all the optically active ingredients and not of the sucrose alone.

Wiley's Temperature Correction Table. Wiley[179] has prepared a temperature table for correcting the readings of quartz-wedge saccharimeters which is based upon the variations in the Ventzke scale reading of normal and fractional normal weights of pure sucrose. This table has a range from 75° V. to 100° V. for temperatures between 4° C. and 40° C.; the corrections are to be subtracted from the observed readings, when the temperature of polarization is below and to be added when the temperature is above that of standardization.

United States Treasury Department Method of Temperature Corrections. The method of temperature corrections devised by the Office of Weights and Measures of the United States Coast and Geodetic Survey, and adopted by the United States Treasury Department for use in the Custom-House laboratories, consists in increasing or diminishing the saccharimeter reading of each sugar solution by the variation in reading which a standard quartz plate shows from the computed sugar value of this plate for the temperature of observation.

The following report gives the temperature corrections in sugar degrees for a quartz control plate tested by the United States Bureau of Standards.

[178] For testimony in this case see "Transcript of Record," U. S. Supreme Court, the American Sugar Refining Company, *vs.* The United States.

[179] *J. Am. Chem. Soc.*, 21, 568 (1899).

DEPARTMENT OF COMMERCE AND LABOR, BUREAU OF STANDARDS, WASHINGTON

Accompanying Report of Temperature Corrections in Sugar Degrees for Quartz Control Plate 233–B.S. 1910

°C.	Sugar Value	°C.	Sugar Value	°C.	Sugar Value	°C.	Sugar Value
13.0°	90.04°	20.0°	90.25°	25.0°	90.40°	30.0°	90.55°
14.0	90.07	20.5	90.27	25.5	90.42	30.5	90.57
15.0	90.10	21.0	90.28	26.0	90.43	31.0	90.58
16.0	90.13	21.5	90.30	26.5	90.45	31.5	90.60
17.0	90.16	22.0	90.31	27.0	90.46	32.0	90.61
17.5	90.18	22.5	90.33	27.5	90.48	32.5	90.63
18.0	90.19	23.0	90.34	28.0	90.49	33.0	90.64
18.5	90.21	23.5	90.36	28.5	90.51	34.0	90.67
19.0	90.22	24.0	90.37	29.0	90.52	35.0	90.70
19.5	90.24	24.5	90.39	29.5	90.54	36.0	90.73

If the polarization temperature is above 20° C., add to the reading the difference between the reading of the plate and the sugar value of the plate at the polarization temperature shown by the above table. If the polarization temperature is below 20° C., subtract the correction.

It will be noted from this table that the variation of 0.030° V. per 1° C., for the reading of a normal weight of pure sucrose, is applied without change to a plate testing 90.25° V. at 20° C. The true temperature correction for a sucrose solution reading 90.25° V. upon the saccharimeter, of course, would be $0.030 \times 0.9025 = 0.027$ per 1° C. The correction table is strictly true, therefore, only for sugar solutions polarizing 100° V. at 20° C. It would be wrong in principle to apply such corrections to sucrose solutions testing 80° V. or 50° V. or 20° V. since in the latter instances the corrections are only 80 per cent, 50 per cent, and 20 per cent, respectively, of the correction for a 100° V. sucrose solution. The correction formula $V^{20°} = V^t \{1 + 0.0003\ (t - 20)\}$ is, therefore, to be preferred when it is desired to correct the polarizations of pure sucrose solutions for change in temperature. The National Bureau of Standards has since adopted this procedure in its reports on standardized quartz plates.

Errors Involved in Use of Saccharimeter Temperature Corrections. The probable errors involved in the use of the above methods for correcting polarizations may be seen from the following diagram (Fig. 201), which gives the correction for pure sucrose solutions, and the approximate corrections for solutions of sugar-beet and sugar-cane products (according to results obtained by Browne[180]), to be applied to the readings of the Ventzke scale for 1° C. increase in temperature.

[180] *J. Ind. Eng. Chem.*, 1, 567 (1909).

It will be seen that the correction for beet products is much nearer the correction for pure sucrose than that for cane products. This is due to the fact that raw cane products contain a larger amount of fructose, the change in specific rotation of which towards the right, as

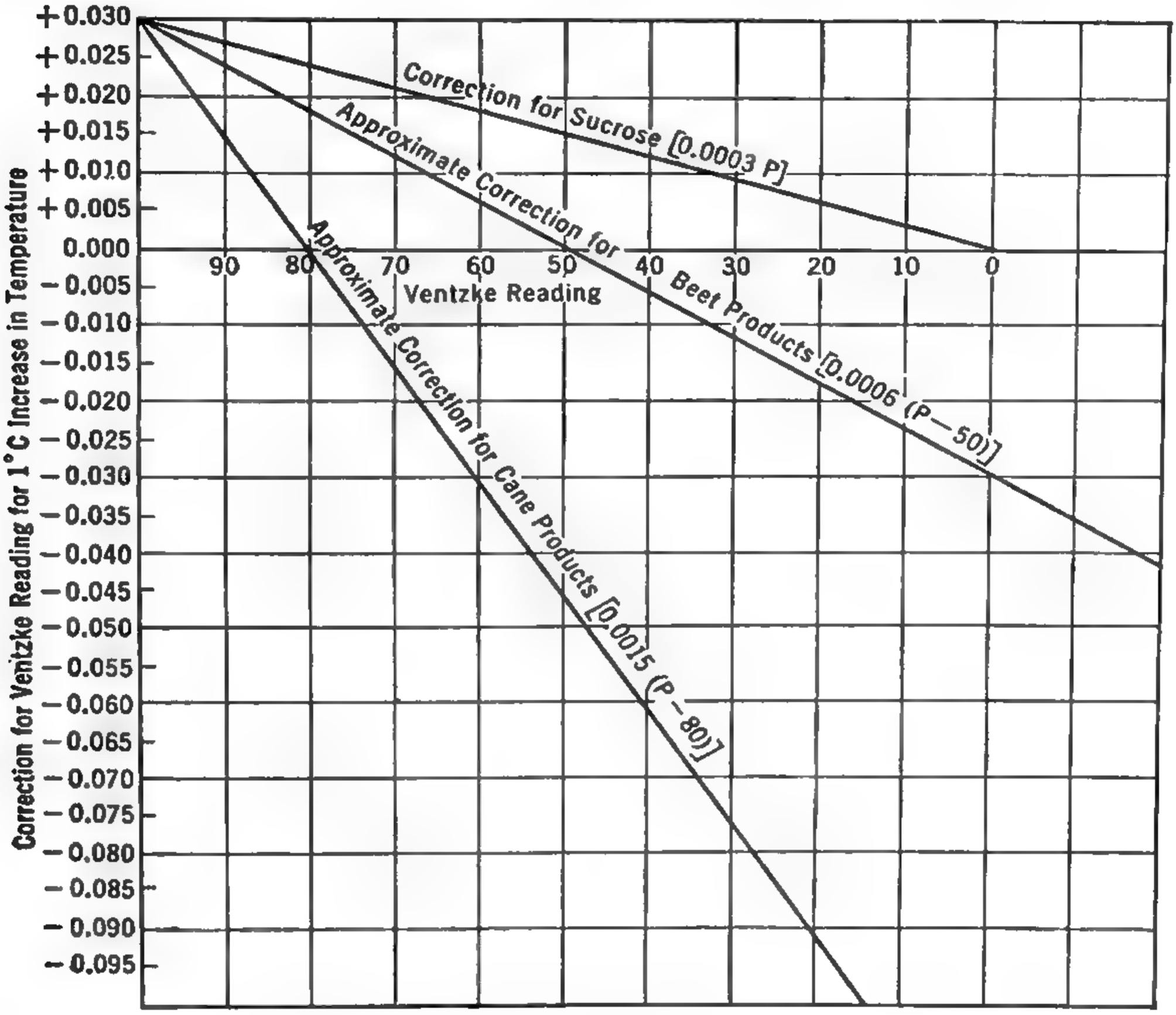


FIG. 201. Diagram for correcting polarizations of sugar products for changes in temperature.

the temperature increases, compensates to a greater or less degree the change in specific rotation of sucrose towards the left. This is made more evident in Table LXII, which gives the polarization and composition of various grades of raw cane sugar.

Raw sugars can be regarded as simple mixtures of sucrose crystals and molasses, and the results in the second part of the table calculated for various theoretical mixtures of sucrose and exhausted cane molasses agree closely with those observed for the different raw sugars.

The observations by Browne in Table LXII have also been con-

firmed by Wiley and Bryan,[181] who obtained very similar figures upon different grades of raw cane sugar.

TABLE LXII

Effect of Increase in Temperature upon the Polarization of Sugar-Cane Products, Browne[182]

No.	Description of Sugar	Polarization	Sucrose	Invert Sugar	Water	Ash	Organic Non-Sugar by Difference	Change in Polarization for 1° C. Increase	
								Found	By Formula 0.0003 *P*
			per cent	per cent	per cent	per cent	per cent		
1	Java.....	98.55	98.74	0.64	0.19	0.21	0.22	−0.0311	−0.0296
2	Peru.....	97.45	97.61	0.52	0.45	0.46	0.96	−0.0301	−0.0292
3	Cuba.....	97.15	97.38	0.78	1.03	0.31	0.50	−0.0276	−0.0291
4	San Domingo..	96.15	96.61	1.53	0.85	0.48	0.53	−0.0230	−0.0288
5	Cuba.....	94.50	95.05	1.83	1:97	0.67	0.48	−0.0212	−0.0287
6	Cuba.....	93.75	94.44	2.29	1.83	0.55	0.89	−0.0160	−0.0281
7	Philippine..	89.20	90.59	4.63	2.11	1.27	1.40	−0.0110	−0.0268
8	Louisiana...	87.60	89.00	4.67	2.30	3.17	0.86	−0.0106	−0.0263
9	Philippine..	82.40	84.64	7.45	3.49	1.85	2.57	0.0000	−0.0247
10	Louisiana...	79.65	81.69	6.80	4.84	4.21	2.46	+0.0068	−0.0239
11	Cuba.....	67.70	71.05	11.18	6.70	3.75	7.32	+0.0286	−0.0203
	Louisiana molasses*	20.06	29.58	30.09	23.62	8.24	8.47	+0.1120	−0.0060

Calculated Mixtures of Sucrose and Cane Molasses

Sucrose, per cent	Molasses, per cent								
95	5	96.00	96.50	1.50	1.10	0.40	0.50	−0.0229	−0.0288
90	10	92.00	93.00	3.00	2.20	0.80	1.00	−0.0158	−0.0276
85	15	88.00	89.50	4.50	3.30	1.20	1.50	−0.0087	−0.0264
80	20	84.00	86.00	6.00	4.40	1.60	2.00	−0.0016	−0.0252
75	25	80.00	82.50	7.50	5.50	2.00	2.50	+0.0055	−0.0240
70	30	76.00	79.00	9.00	6.60	2.40	3.00	+0.0126	−0.0228

* Average of four samples.

The effect of temperature upon the polarization of American beet sugar and molasses is shown in Table LXIII.

[181] *Z. Ver. deut. Zucker-Ind.*, **59**, 916 (1909).
[182] *J. Ind. Eng. Chem.*, **1**, 567 (1909).

TABLE LXIII

EFFECT OF INCREASE IN TEMPERATURE UPON THE POLARIZATION OF SUGAR-BEET PRODUCTS, Browne[183]

No.	Product	Polarization	Sucrose	Raffinose	Invert Sugar	Water	Ash	Organic Non-Sugar by Difference	Change in Polarization for 1° C. Increase	
									Found	Formula 0.0003 *P*
		per cent	per cent	per cent	per cent	per cent	per cent	per cent		
1	Beet sugar.	91.25	.....	....	....	.....	....	.....	−0.0276	−0.0274
2	Beet sugar.	86.60	.....	....	....	.....	....	.....	−0.0263	−0.0260
3	Beet sugar.	85.50	.....	....	....	.....	....	.....	−0.0214	−0.0257
4	Beet molasses*	51.22	48.13	1.72	0.94	19.86	7.62	21.74	−0.0053	−0.0154

Calculated Mixtures of Sucrose and Beet Molasses

Sucrose, per cent	Molasses, per cent									
90	10	95.00	94.80	0.15	0.10	2.0	0.75	2.20	−0.0275	−0.0285
80	20	90.00	89.60	0.30	0.20	4.0	1.50	4.40	−0.0250	−0.0270
70	30	85.00	84.40	0.45	0.30	6.0	2.25	6.60	−0.0225	−0.0255
60	40	80.00	79.20	0.60	0.40	8.0	3.00	8.80	−0.0200	−0.0240

* Average of three samples.

It will be seen from the above that the temperature formula $P^{20} = P^t\,[1 + 0.0003\,(t - 20)]$, or the corresponding corrections of the Wiley table, can be applied without serious error to practically all grades of beet sugar and to those grades of cane sugar polarizing over 96. As the polarization of raw cane sugars falls below 96, and the percentage of invert sugar (or fructose) increases, the effect of change in temperature upon the rotation of the latter begins to lower appreciably the temperature coefficient for the rotation of sucrose until, at a point about 80° V., the two influences — that of the temperature upon the fructose and other impurities and that of the temperature upon the sucrose and quartz wedges of the instrument — exactly counterbalance each other.[184] Under these conditions a sugar will polarize the same at all temperatures. Below 80° V. the temperature coefficient for the rotation of the sucrose in raw cane sugars is usually more than counterbalanced, the result being that the polarization of these sugars *increases* with elevation of temperature. This increase continues, as

[183] *J. Ind. Eng. Chem.*, 1, 567 (1909).

[184] The calculation upon p. 197 shows that the proportion of fructose to sucrose for equilibrium between their temperature coefficients is 3.32 to 100.0.

the polarization diminishes (the percentage of fructose and other impurities being greater), until, at a polarization of about +20 for exhausted cane molasses, an increase of 1° C. in temperature causes an increase of over 0.1° V. in the saccharimeter reading.

Correction of Polarizations for the Combined Influence of Temperature upon the Rotation of Sucrose and Invert Sugar. Since the ingredient of sugar products, whose polarization is most susceptible to the influence of temperature, is invert sugar, a more accurate method of correcting saccharimeter readings is to combine the temperature coefficients of sucrose and invert sugar as by the formula:[185]

$$P^{20} = P^t + 0.0003\,S\,(t - 20) - 0.0045\,I\,(t - 20)$$

in which P^t is the polarization at $t°$ C., S the percentage of sucrose, and I the percentage of invert sugar.

If the percentage of invert sugar is unknown the temperature correction for converting polarizations to 20° C. may be determined approximately by the following empirical equations:

For cane products, $P^{20} = P^t + 0.0015\,(P^t - 80)\,(t - 20)$
For beet products, $P^{20} = P^t + 0.0006\,(P^t - 50)\,(t - 20)$

Such formulas as the above, though more accurate than corrections which are based upon the temperature coefficients of pure sucrose, fail to give accurate results upon many individual products whose composition differs from that of the average type. The extent of this error may be seen from the following results of Bryan,[186] who compared 4117 polarizations of raw sugars, made at ordinary temperature and corrected by the above formula for cane products, with the polarizations of the same sugars obtained at 20° C.:

Results within ± 0.05	of the	20° C. polarization 48.9%
Results from 0.05 to 0.15	higher than the	20° C. polarization 16.4%
Results from 0.05 to 0.15	lower than the	20° C. polarization 17.4%
Results from 0.15 to 0.25	higher than the	20° C. polarization 6.2%
Results from 0.15 to 0.25	lower than the	20° C. polarization 5.9%
Results from 0.25 to 0.35	higher than the	20° C. polarization 2.1%
Results from 0.25 to 0.35	lower than the	20° C. polarization 1.7%

[185] Horne (*Facts About Sugar,* 7, 53) gives the formula: $P^{20} = P^t + 0.0003\,S\,(t - 20) - 0.00812\,F\,(t - 20)$, where F is the percentage of fructose in the sugar. The factor 0.00812 had been calculated by Browne (*Ind. Eng. Chem.,* 1, 567) from the formula of Jungfleisch and Grimbert for the specific rotation of fructose; later investigations have shown it to be too low (see pp. 196 and 272).

[186] *J. Assoc. Official Agr. Chem.,* 4, 328 (1921).

Results from 0.35 to 0.45	higher than the 20° C. polarization	0.5%
Results from 0.35 to 0.45	lower than the 20° C. polarization	0.5%
Results over 0.45	higher than the 20° C. polarization	0.3%
Results over 0.45	lower than the 20° C. polarization	0.1%

The average of the corrected results was within 0.01 of that obtained for the 20° C. polarizations.

Similarly, Douwes Dekker[187] found for Java raw sugars of 97 to 99 polarization an average temperature coefficient of 0.0003, but in individual cases it varied from 0.00006 to 0.00063. The discrepancies could not be explained by the effect of the invert sugar alone.

Polarization at Constant Temperature. It is evident from the foregoing that the method of applying temperature corrections established for pure sucrose to the polarization of sugar products in general is faulty. Since it is impossible to devise a simple reliable method of temperature corrections that can be applied to the polarization of all kinds of substances, the one means of securing uniformity and accuracy in saccharimetric work is to make all polarizations at the temperature at which the instruments are standardized. Customhouse laboratories, arbitration laboratories, and all other laboratories, upon the results of which great interests are involved, should be equipped with cooling and warming apparatus for maintaining a uniform standard temperature throughout the year.

The New York Sugar Trade Laboratory was the first testing laboratory in the United States to follow out the requirements of the International Commission for Uniform Methods of Sugar Analysis and make all polarizations at 20° C. The laboratory room and polarizing cabinet used for this purpose are insulated. The cooling equipment installed originally[188] for use during warm weather consisted of a small ammonia compressor and a ventilating fan which drew outside air over the expansion coils and blew it into the room through a duct with adjustable openings. The air was partly recirculated, and the temperature was regulated by means of rheostats controlling the speed of the compressor and of the ventilating fan. During the winter months the room was kept at the required temperature by steam heat and an auxiliary gas heater.

After twenty-eight years of satisfactory service this constant-temperature equipment was replaced by a system which automatically maintains the correct temperature both summer and winter. Cold air is provided by a Freon (CCl_2F_2) compressor, and warm air by electric

[187] *Arch. Suikerind.*, 37, II, 909 (1929).

[188] For a full description of the equipment and its operation see Browne, *Orig. Com. 8th Intern. Congr. Appl. Chem.*, 25, 519 (1912).

strip heaters placed in the ventilating duct near the fan. The temperature is regulated by a thermostat which, through relays, actuates two electromagnetic switches and turns on and off either the compressor motor or the electric strip heaters. A part of the constant-temperature room is shown in Fig. 202. The ventilating duct is near the ceiling, and the saccharimeter cabinet (Fig. 167) is behind the black curtain in the right background. The expansion coils and ventilating fan are in a box hung from the ceiling in the saccharimeter cabinet. The compressor and accessories, Fig. 203, are in a room adjoining the constant-temperature room, separated from it by an insulated wall.

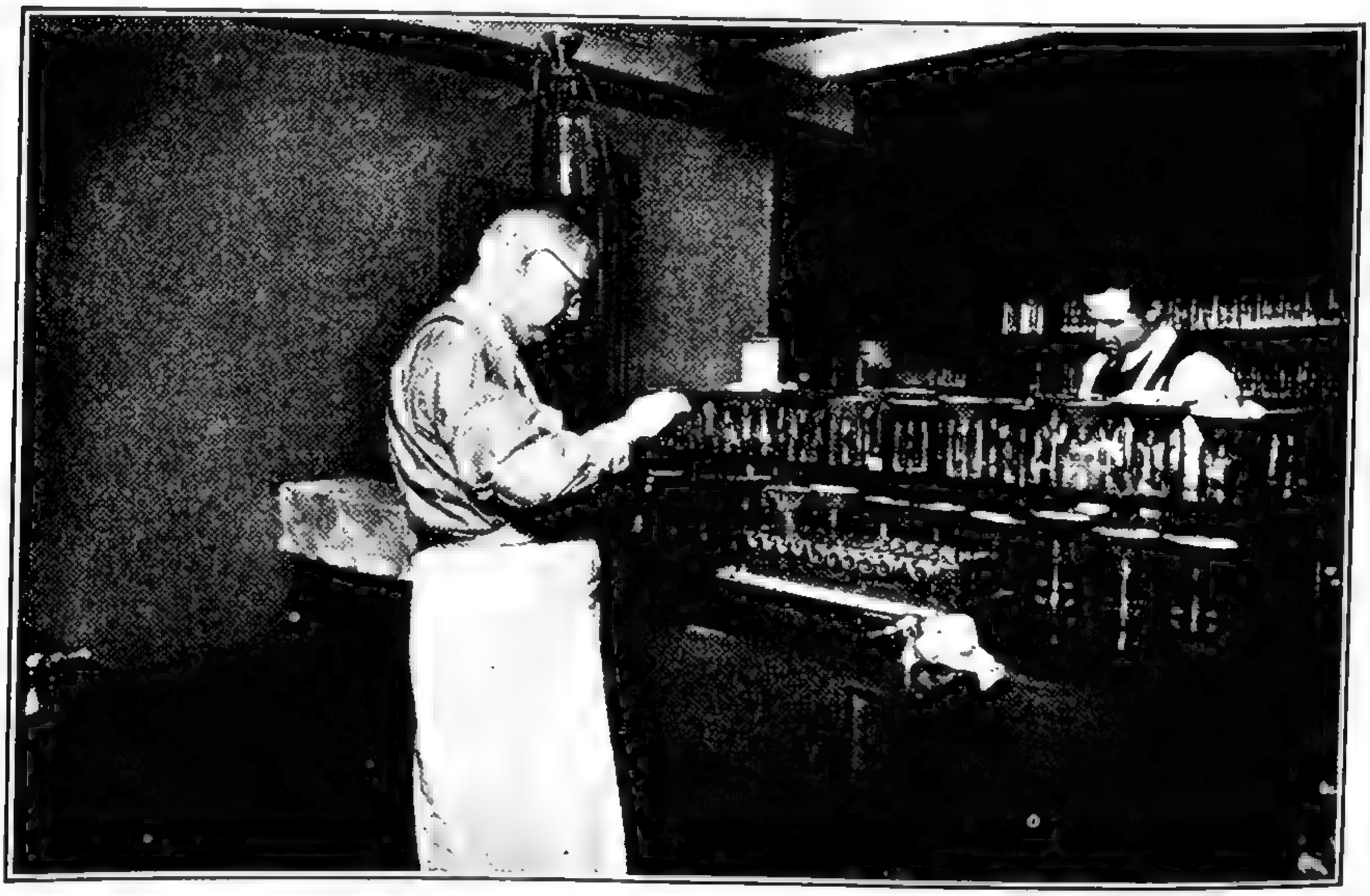


FIG. 202. Showing portion of constant-temperature room (New York Sugar Trade Laboratory).

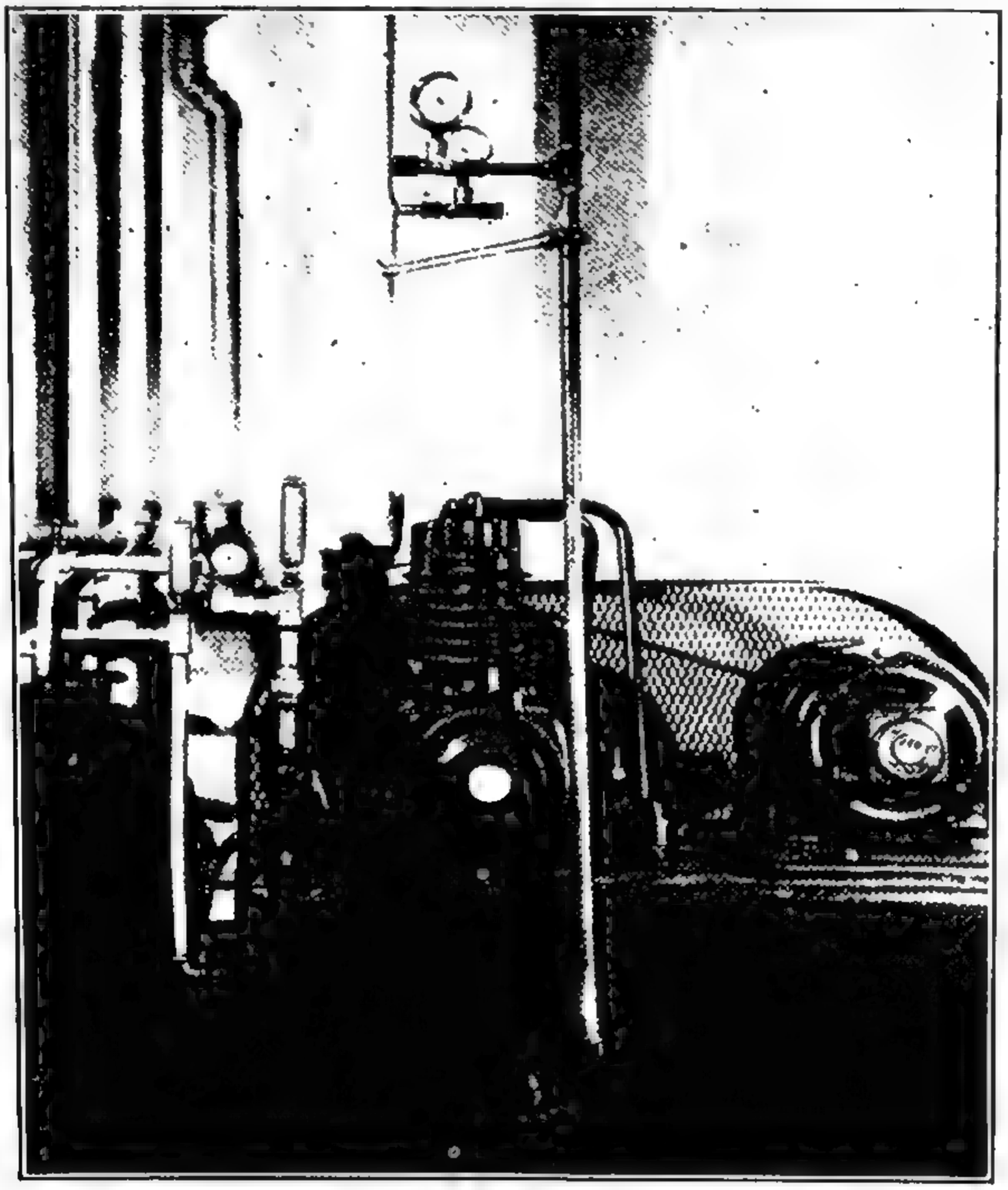
TREATMENT OF ERRORS IN SACCHARIMETRIC ANALYSIS

The effect of the combined influences of the various small errors in saccharimetric analysis is an important question especially in the valuation of commercial products. Browne[189] has named the following twelve more common errors in the polarization of sugars: (1) loss of moisture in mixing, (2) loss of moisture in weighing, (3) error in normal weights, (4) volume of lead precipitate, (5) precipitation of fructose,

[189] *Louisian Planter*, **54**, 26 (1915), where the treatment of errors in saccharimetry is fully discussed.

(6) error in volume of flasks, (7) imperfect mixing of contents of flasks, (8) evaporation in filtering, (9) error in length of polariscope tubes, (10) omission of bichromate cell, (11) variations in temperature, (12) error in scales of saccharimeters.

Of the above errors those of calibration (items 3, 6, 9, and 12) should be mutually compensating. The slight permissible errors, or tolerance, in weights, flasks, tubes, and saccharimeters should as far as possible be so evenly balanced above and below the true value that the general



(*New York Sugar Trade Laboratory.*)

FIG. 203. Refrigerating machine for constant temperature polarization.

average of the laboratory's results may be correct. It would be wrong, for example, for every flask in a lot to be 0.03 ml. above the true capacity although permissible in a single flask. The remaining eight manipulative errors of saccharimetry are not mutually compensating, as each one tends for the most part to produce an increase in polariza-

tion. The practice, therefore, of disregarding any one error because small may become pernicious if generally applied. Seven errors of +0.03 when combined will produce a total error of +0.2. The following three rules are given for reducing the final aggregate error:

First. Keep all errors in calibration of apparatus as low and as evenly balanced as possible.

Second. Eliminate all preventable errors, however small.

Third. Reduce all unavoidable errors to the minimum.

The final residual error in the carefully conducted polarization of raw cane sugars of 96 test is estimated by Browne as follows:

Error Due to	Sugar Degrees
Evaporation in mixing	+0.010
Evaporation in weighing out	+0.005
Volume of lead precipitate	+0.090
Precipitation of fructose	+0.015
Total error	+0.120

The error of —0.105 reported by Bates and Jackson in the German sugar scale is thus counterbalanced by certain errors of method.

Personal Equation. It is a common observation that certain chemists tend always to read sugar solutions higher and others lower than a general average. These differences are mostly due to the varying sensibility of different eyes to the faint inequalities of color in the two parts of the field produced by the slightly unequal rotation of rays of light that are not perfectly monochromatic (see p. 182). For this reason Landolt[190] states, "Measurements can never be made with a saccharimeter without perceptible systemic errors." From experiments with a control tube Browne[191] noted between different observers one-way differences of 0.04 sugar degree. Changing the half-prism of a Lippich polarizer to the opposite side of the field was found to reverse the order of personal equation. A saccharimeter upon which every observer can obtain exactly the same reading of a sugar solution is an impossibility, and this is one explanation of the disputes which arise concerning the correctness of normal weights.

Limits of Variation. The permissible variation between the saccharimetric determinations of two chemists should not ordinarily exceed 0.2 and under careful control of conditions may with uniform samples be kept as low as 0.1 sugar degree. The frequencies of magni-

[190] "Das optische Drehungsvermögen," 2nd ed., p. 378, 1898..

[191] *J. Ind. Eng. Chem.*, **12**, 796 (1920).

tude are represented by a bell-shaped curve from which the theoretical variations can be calculated. The following examples are given by Browne:[192]

	For Ordinary Control		For Careful Control	
	Theory	500 Observations	Theory	500 Observations
	per cent	per cent	per cent	per cent
Polarizations agreeing	19	20.0	30.6	29.4
Polarizations differing by ±0.05	32	31.8	44.4	47.2
Polarizations differing by ±0.10	24	23.4	22.2	21.4
Polarizations differing over ±0.10	25	24.8	2.8	2.0

[192] *Louisiana Planter*, 54, 29 (1915).

CHAPTER X

METHODS OF INVERT OR DOUBLE POLARIZATION

The methods of direct polarization, as previously explained, give percentage of sucrose only in the absence of other optically active substances. To determine the percentage of sucrose when other optically active substances are present, the method of inversion or double polarization is generally used, the principle of which may be understood from the following.

Law of Inversion. When a solution of sucrose is acted upon by some inverting agent, such as an acid or the enzyme invertase, the sucrose molecule is broken up or inverted, giving rise, by the addition of one molecule of water, to one molecule each of glucose and fructose, the mixture of these two sugars in equal amounts being termed invert sugar. This reaction, known as hydrolysis or inversion, is expressed by the following equation:

$$\underset{\text{Sucrose (342)}}{C_{12}H_{22}O_{11}} + \underset{\text{Water (18)}}{H_2O} \rightarrow \underbrace{\underset{\text{Glucose (180)}}{C_6H_{12}O_6} + \underset{\text{Fructose (180)}}{C_6H_{12}O_6}}_{\text{Invert Sugar (360)}}$$

Although this equation involves the disappearance of one molecule of water with each molecule of sucrose, and the reaction is therefore apparently bimolecular, inversion by acid, that is by hydrogen ions, nevertheless follows the unimolecular reaction law, according to which the rate of the reaction

$$\frac{dx}{dt} = k\,(a - x)$$

where a is the original amount of sucrose present, x the quantity inverted at the end of time t after the commencement of the inversion, dx the infinitesimal quantity inverted during the infinitesimal time interval dt, and k a constant which is termed the velocity coefficient of the inversion. Integration gives the following value for

$$k = \frac{1}{t} \log \text{nat.} \frac{a}{a - x}$$

or, changing from natural to common logarithms, ($\log_{10} = 0.4343 \log$ nat.),

$$k = \frac{1}{0.4343\,t} \log_{10} \frac{a}{a - x}$$

For purposes of comparison it is quite customary to express k on the basis of common rather than natural logarithms.

The unimolecular course of inversion by hydrogen ions is usually explained by the large excess of water present and the consequent slight diminution in the total active mass of water. Another explanation is offered, according to modern theory,[1] by the tendency of sucrose to form hydrates in aqueous solution; the oxonium ion, H_3^+O, attaches itself to oxygen atoms in the sucrose molecule, and the complex thus formed at once decomposes again at the rate measured by the rate of inversion. The mechanism may be illustrated by the following formulas:

$$C_{12}H_{22}O_{11},\ xH_2O + H^+ \rightarrow C_{12}H_{22}O_{11},\ xH_2O \cdot H^+ \rightarrow$$
$$2C_6H_{12}O_6 + (x - 1)\ H_2O + H^+$$

The chemical equation for the inversion of sucrose shows that 1 part of sucrose is converted into $360 \div 342 = 1.05263$ parts of invert sugar. Calling the specific rotation of sucrose $+66.5$ at 20° C., and that of the half-normal weight of invert sugar at the same temperature -20.28 (p. 270), the relation of the optical activity of 1 part of sucrose before inversion to that after inversion will be $66.5 : 1.05263\ (-20.28) = 66.5 : -21.3473$, amounting to a decrease of 87.8473 in specific rotation. This decrease for 1 degree of the saccharimeter scale would therefore be $87.8473 \div 66.5 = 1.3210$. The general law of inversion as applied to the determination of sucrose may then be stated as follows:

The total decrease in the saccharimeter reading at 20° C., of the half-normal weight of product after inversion, divided by 1.3210, and then multiplied by 2, gives the percentage of sucrose when no other optically active ingredient is hydrolyzed and when the inverting agent produces no change in the specific rotation of the other optically active constituents present.

The enzyme invertase fulfills most perfectly the conditions above named, and when this is used as the inverting agent the percentage of sucrose in mixtures with glucose, fructose, invert sugar, maltose, milk sugar, etc., may be determined very closely by use of the factor 1.3210, provided that the concentration of dry substance in the solution is approximately 13 g. in 100 ml. The inverting agent most commonly used in optical analysis is not invertase, however, but hydrochloric acid, the presence of which, as shown on p. 281, has a most pronounced influence in increasing the specific rotation of fructose. When hydrochloric acid is used for inverting, the factor 1.3210 must be modified according to the amount of acid used for inverting, the concentration of the sugar

[1] Pearce and Thomas, *J. Phys. Chem.*, 42, 455 (1938).

solution, and the manner of conducting the inversion. The extreme susceptibility of fructose to changes in specific rotation and composition makes it necessary in any method of inversion to adhere rigidly to the prescribed rules.

POLARIMETRIC SUCROSE DETERMINATION BY SIMPLE INVERSION WITH HYDROCHLORIC ACID

The inversion method for determining sucrose in the presence of other optically active substances was first devised by Biot[2] in 1842 for the purpose of analyzing the juice of maize stalks. His method of calculation was according to the formula $S = \left(\frac{1 - \frac{i}{p}}{1 - \frac{I}{P}}\right) p$, in which S is the percentage of sucrose; i and p are the invert and direct polarizations, respectively, of the juice; and I and P the invert and direct polarizations, respectively, of pure sucrose under the same conditions as the analysis. The quantity $1 - I/P$, according to Biot's process of inversion with hydrochloric acid, was found to be 1.3867.

After the invention of Soleil's saccharimeter Biot's pupil Clerget[3] in 1846 gave more careful study to the details of the inversion process and was the first to standardize the method which afterwards bore his name. Clerget found that a solution of the French normal weight of pure sucrose in 100 cc., reading +100° upon the saccharimeter, gave after inversion with hydrochloric acid a reading of —44° at 0° C. or —34° at 20° C. The total difference between the readings before and after inversion, correcting for the influence of temperature, is expressed by the quantity

$$100 - (-44) - \frac{t}{2} = 144 - \frac{t}{2} \qquad (1)$$

t being the temperature of the inverted solution at polarization.

If D represents the algebraic difference $(P - P')$ between the direct polarization (P) and the invert polarization (P') of a given product, then the percentage (S) of sucrose by Clerget's formula is expressed by the equation $S = \frac{100\,D}{144 - \frac{t}{2}}$. If the invert polarization is made at 20° C.

[2] *Compt. rend.*, **15, 537 (1842).**

[3] "Analyse des sucres et des substances saccharifères," paper presented before la Société d'encouragement pour l'industrie nationale, Oct. **14, 1846.** See also *Compt. rend.*, **16, 1000 (1843); 22, 1138 (1846); 23, 256 (1846); 26, 240 (1848); *Ann. chim. phys.* [3], 26, 175 (1849).**

the equation becomes $S = \frac{100\,D}{134}$ or $\frac{D}{1.34}$. The factor 1.34 is considerably greater than the factor 1.3210 for pure aqueous solutions of invert sugar.

Tuchschmid,[4] who subjected the Clerget process to an exhaustive analysis, arrived at the following formula:

$$S = \frac{100\,D}{144.16035 - 0.50578\,t}$$

The original Clerget formula does not differ sufficiently from this to warrant the greater labor of calculation involved in the use of the long decimals.

If the direct and invert readings are made upon a polarimeter with circular degrees the Clerget formula would be, for the German normal weight (1° sugar scale = 0.34657 circular degrees),

$$\frac{100\,D}{0.34657\,(144 - 0.5\,t)} = \frac{100\,D}{49.906 - 0.173\,t}$$

for the French normal weight (1° sugar scale = 0.21667 circular degrees):

$$\frac{100\,D}{0.21667\,(144 - 0.5\,t)} = \frac{100\,D}{31.200 - 0.108\,t}$$

One gram of sucrose dissolved in 100 milliliters gives a direct reading of $\frac{34.657}{26.026} = 1.332$ circular degrees and an invert reading of $-\frac{15.249}{26.026} = -0.5859$ circular degrees at 0° C; the grams of sucrose (C) in 100 ml. of any solution may be found from the polarimeter reading before and after inversion by the equation

$$C = \frac{P - P'}{\frac{(49.906 - 0.173\,t)}{26.026}} = \frac{P - P'}{1.9175 - 0.0066\,t}$$

The Clerget formulas, given above, are to be employed only when the following method of inversion prescribed by Clerget is followed. After taking the direct polarization (p. 304), the clarified solution remaining is filled up to the 50-ml. graduation mark of a flask graduated at 50 and 55 ml.; concentrated hydrochloric acid is then added to the 55-ml. mark, a thermometer is inserted, and the flask slowly warmed until the temperature reaches 68° C., 15 minutes being taken in the heating. The solution is then quickly cooled, filtered if necessary, and polarized as nearly as possible at the original temperature of making up

[4] *Z. Ver. deut. Zucker-Ind.*, 20, 649 (1870).

to volume. The polariscope reading for a 200-mm. tube of solution must be increased by $\frac{1}{10}$ to correct for the dilution with acid. The reading of the inverted solution is sometimes made in a 220-mm. tube, when no correction for dilution is needed.

In carrying out the inversion special attention must be paid to all details. If the temperature of 68° C., or the time of 15 minutes, is exceeded, a partial destruction of fructose may result; if the temperature of 68° C. is not reached, or if the time of heating is less than 15 minutes, some of the sucrose may escape inversion. Care must also be taken to maintain a constant temperature in the polarization tube during the reading. Even a slight warming of the tube, as from handling, will affect the observation. A polarization tube provided with a jacket for circulation of water at the desired temperature is very desirable for polarizing inverted solutions. (See Fig. 153.) The temperature must be read to 0.1° C.

Herzfeld's Modification of the Clerget Method. The original method of Clerget has been variously modified from time to time in order to diminish the danger of destroying fructose and to secure better uniformity of conditions. The inversion method of Herzfeld,[5] devised in 1888, is carried out as follows. A solution of the German normal weight in 100 ml. is prepared. A part of this solution is used for the direct polarization (P). Another 50-ml. portion of the solution is pipetted into a 100-ml. flask, diluted with 25 ml. of water, and 5 ml. of hydrochloric acid of sp. gr. 1.188 is added. The flask is placed in a water bath heated to 72 to 73° C. As soon as the solution in the flask reaches 69° C., which requires 2.5 to 5 minutes, the solution is kept at this temperature for exactly 5 minutes longer, the flask being gently rotated at frequent intervals. The flask is then cooled as quickly as possible to 20° C., the volume is completed to the mark with water and the solution read in the saccharimeter. The reading, multiplied by 2, gives P'. The percentage of sucrose S is calculated by the formula:

$$S = \frac{100\,(P - P')}{132.66 - 0.5\,(t - 20)}$$

The Herzfeld inversion procedure is still widely employed although it has been proved that it does not give correct results in the presence of substances whose rotation is affected by hydrochloric acid. As Herzfeld's inversion procedure allowed too much latitude in operation, Schrefeld[6] has standardized it most carefully, and the Association of Official Agricultural Chemists has adopted his modification as an official

[5] *Z. Ver. deut. Zucker-Ind.*, **38**, 699 (1888).
[6] *Z. Ver. deut. Zucker-Ind.*, **70**, 402 (1920).

method, but only for products not containing much fructose. The directions are as follows:[7]

Pipette a 50-ml. portion of the solution into a 100-ml. flask and add 25 ml. of water. Then add, little by little, while rotating the flask, 10 ml. of hydrochloric acid (*d* 1.1029 at 20°/4° or 24.85° Brix at 20° C.). Heat a water bath to 70° C. and regulate the burner so that the temperature of the bath remains approximately at that point. Place the flask in the water bath, insert a thermometer, and heat with constant agitation until the thermometer in the flask indicates 67°. (This preliminary heating period should require from 2.5 to 2.75 minutes.) From the moment the thermometer in the flask indicates 67°, leave the flask in the bath for exactly 5 minutes longer, during which time the temperature should gradually rise to about 69.5°. Plunge the flask at once into water at 20°. When the contents have cooled to about 35°, remove the thermometer from the flask, rinse it, and fill almost to the mark. Leave the flask in the bath at 20° for at least 30 minutes longer and finally make up exactly to volume. Mix well and polarize the solution in a 200-mm. tube provided with a lateral branch and a water jacket, maintaining a temperature of 20°. This reading must be multiplied by 2 to obtain the invert reading P'. If it is necessary to work at a temperature other than 20°, which is permissible within narrow limits, the volumes must be completed and both direct and invert polarizations must be made at exactly the same temperature.

The Herzfeld constant of 132.66 at 20° C., corresponding to 142.66 at 0°C., is generally admitted to be too low. Steuerwald[8] found 133.05, and Staněk[9] 132.95, while Jackson and Gillis[10] computed from their measurements a value between 133.06 and 133.11. Schrefeld obtained 133.0 by the procedure outlined above, and this figure was confirmed by Browne.[11] Zerban and coworkers[12] reported 132.97 ± 0.02. The average of all these figures is 132.99, which may be rounded off to 133.0, and the Association of Official Agricultural Chemists has accepted this value, resulting in the following Clerget formula:

$$S = \frac{100\,(P - P')}{143 - 0.5\,t}$$

where P is obtained by diluting 50 ml. of the original solution with water to 100 ml., and multiplying the reading by 2.

[7] " Methods of Analysis, A. O. A. C.," 5th ed., p. 494, 1940.
[8] *Arch. Suikerind.*, **21**, 1383 (1913).
[9] *Z. Zuckerind. Böhmen*, 38, 296 (1913/14).
[10] *Bur. Standards Sci. Paper* 375, p. 153, 1920.
[11] *J. Ind. Eng. Chem.*, 13, 794 (1921).
[12] *J. Assoc. Official Agr. Chem.*, 8, 384 (1925).

Staněk[13] found that, if the solution which has been inverted at 67 to 70° C. is quickly cooled to 20° and read immediately, a lower result is found than if the solution is permitted to stand for 15 to 20 minutes, after which the rotation becomes constant. This indicates a lag in rotation change with change of temperature similar to the mutarotation with change in concentration. This cause of error is guarded against in Schrefeld's method, and the chemist should always keep it in mind.

Results by Jackson and Gillis[14] show that there is a slight decomposition of invert sugar when the inversion is carried out at temperatures near 70° C. But if the temperature is reduced to 60°, with a total heating period of 9 minutes, there is no destruction of invert sugar. When 13 g. of sucrose in 70 ml. solution is inverted with 10 ml. of hydrochloric acid of d_4^{20} 1.1029 under the conditions just stated, and then diluted to 100 ml., the Clerget constant is 133.25 at 20° C. Obviously, with departures in the conditions of concentration, amount of acid, volume, temperature, time, and other details, variations in the constant are to be expected.

The standardization of the conditions of inversion and the evaluation of the Clerget divisor still remain to be fixed by international agreement.

Effect of Temperature on the Clerget Constant. The temperature coefficient of 0.5, based on the work of Clerget and of Tuchschmid, considers only the effect of temperature on the rotation of the invert sugar, but not that on the rotation of sucrose, which at that time was considered to be negligible. Prinsen Geerligs[15] called attention to this point and proposed that a correction be applied also for the latter effect, amounting to —0.03 for a rise of 1°C. and a direct reading of 100° V. The divisor for the Herzfeld formula should accordingly read 142.66 — $0.5\,t - 0.03\,(t - 20)$. This may also be written 142.66 + 0.6 — $0.53\,t$, or 143.26 — $0.53\,t$. In order to simplify the calculations, Prinsen Geerligs gave a table of Clerget divisors based on these formulas. Subsequently Steuerwald[16] published a similar table, for the corrected divisor 133.05 at 20° C. These two tables have been misinterpreted in some textbooks on sugar analysis. The temperatures shown in the top line of the tables refer not to that of the saccharimeter, but to that of the inverted solution at the moment of reading, which temperature must be equal to or as nearly as possible equal to the temperature of the solution for the direct reading as well as that of the saccharimeter. The Clerget divisors given in the tables are those corrected for the effect of

[13] *Z. Zuckerind. Böhmen,* **38**, 289 (1913/14).
[14] *Bur. Standards Sci. Paper* **375**, p. 139, 1920.
[15] *Arch. Suikerind.*, **21**, 33 (1913); *Intern. Sugar J.*, **15**, 241 (1913).
[16] *Arch. Suikerind.*, **21**, 1383 (1913); *Intern. Sugar J.*, **16**, 82 (1914).

temperature on the rotation of the sucrose in the solution for the direct reading. From these divisors 0.5 *t* must be deducted to allow for the effect of temperature on the rotation of invert sugar.

Sázavský[17] also made a proposal to combine the two temperature coefficients into one, that is —0.53 *t*. Jackson and Gillis[18] have adopted this coefficient in their tables of Clerget divisors.

Gubbe's equation for the change in the rotation of invert sugar with temperature, between 0 and 30° C. (p. 272), shows that the temperature coefficient is not a linear function of the temperature but increases more rapidly than the temperature change. For a temperature interval of 1° from the normal of 20° C. the coefficient equals 0.484, for an interval of 5° it is 0.495, and for one of 10° it is 0.508.

Vosburgh[19] has shown that the temperature coefficient of the rotation of fructose, and hence of invert sugar, varies also with the concentration. His results, recalculated for the invert sugar formed from the half-normal weight (13 g.) of sucrose, give a coefficient of —0.478; for the quarter-normal weight the coefficient equals —0.466; for the quarter-normal weight of a final cane molasses the coefficient is —0.460. The corresponding combined coefficients for invert sugar and sucrose are —0.508, —0.496, and —0.490, respectively, considerably lower than the figure 0.53 given above. Gillet[20] has reported a coefficient of —0.49 for the invert sugar formed from a half-normal weight of sucrose, which would make a combined coefficient of —0.52. Some unpublished work by Zerban points to the conclusion that for final cane molasses, at quarter-normal concentration, the combined coefficient —0.53 is considerably too high.

In most sugar countries the rounded-off coefficient —0.5 *t* has been retained, a practice which is justified in view of the uncertainty regarding proper temperature corrections at varying concentrations and temperature differences from the normal. This coefficient is therefore used in the Clerget formulas given on succeeding pages.

It has long been a custom to base Clerget divisors on a temperature of 0° C., and this simplifies the formulas. But in actual practice most of the experimental work on the divisors has been done at or near 20° C. This fact, combined with the uncertainty in the temperature coefficient over wide ranges of temperature, makes it preferable to use 20° C. as the basic temperature. It is advisable for the chemist always to work as closely as possible at 20° C., because this will minimize any errors

[17] *Z. Zuckerind. čechoslovak. Rep.*, **48**, 261 (1923/24).

[18] *Bur. Standards Sci. Paper* 375, p. 183 ff., 1920.

[19] *J. Am. Chem. Soc.*, **42**, 1697 (1920); see also Zerban, *J. Am. Chem. Soc.*, **47**, 1104 (1925).

[20] *Z. Ver. deut. Zucker-Ind.*, **64**, 271 (1914).

arising from temperature corrections. The best practice, as in simple polarization, is the use of a constant-temperature room kept at 20° C.

Effect of Concentration on the Clerget Divisor. With solutions of pure sucrose the divisor 133.0 at 20° C. for the Herzfeld-Schrefeld inversion procedure is correct only if the solution to be inverted contains 13 g. sucrose in a final volume of 100 ml. For other concentrations the divisor is different, owing to variations in the specific rotation of invert sugar; the effect of the slight changes in the rotation of sucrose is practically within the limit of error.

The Clerget divisor increases, according to Herzfeld,[21] by 0.0676 for each additional gram of sucrose in 100 ml. solution. At 20° C. the divisor for the Schrefeld procedure equals $133 + 0.0676\ (g - 13)$, where g denotes grams sucrose in 100 ml. final volume of the solution being inverted. The complete formula for any concentration or temperature may then be written

$$S = \frac{100\ (P - P')}{133.0 + 0.0676\ (g - 13) - 0.5\ (t - 20)}$$

where S is per cent sucrose, and P and P' the direct and invert polarization, respectively, calculated to the normal-weight basis.

The following table gives the Clerget constants derived from the above equation, for 1 to 13 g. sucrose in 100 ml., at 20° C. To correct for the effect of temperature, $0.5\ (t - 20)$ must be deducted from the divisors shown.

TABLE LXIV

CLERGET DIVISORS FOR HERZFELD-SCHREFELD PROCEDURE

Grams of Inverted Sucrose in 100 ml.	Divisor, 20° C.	Grams of Inverted Sucrose in 100 ml.	Divisor, 20° C.
1	132.19	8	132.66
2	132.26	9	132.73
3	132.32	10	132.80
4	132.39	11	132.86
5	132.46	12	132.93
6	132.53	13	133.00
7	132.59	..	.....

Steuerwald has reported a slightly higher concentration factor, 0.0717, but Herzfeld's factor has been confirmed by Herles (0.067), by Sázavský (0.0677), and by Jackson and Gillis (0.0676).[22]

[21] *Z. Ver. deut. Zucker-Ind.*, 40, 205 (1890).

[22] More recent investigations by Jackson and others indicate, however, that the coefficient 0.0676 is too low, and is probably nearer 0.08. See Jackson and McDonald, *J. Assoc. Official Agr. Chem.*, 22, 580 (1939).

In applying the inversion method to products which contain besides sucrose also other sugars or non-sugars, for a long time it was believed that the proper divisor to be used in the Clerget formula is that based on the concentration of the sucrose alone. But Browne[23] has shown this to be erroneous, and his results have been confirmed by Zerban and coworkers.[24] It is not the partial sucrose concentration which determines the Clerget divisor, but rather the water concentration, or what amounts to the same thing, the dry-substance concentration.[25] In the analysis of impure products it is only necessary to substitute, in the above formula and table, for g the grams of dry substance in 100 ml. final volume of the solution to be inverted. This can be readily ascertained by determining the refractometric Brix of the solution, and multiplying by the density corresponding to the Brix found. Or the Brix of the original product is determined by the refractometric or densimetric method, and the value found is multiplied by 0.13 to obtain the grams of dry substance in 100 ml. of the half-normal solution of the product.

Formulas and tables are found in the literature and textbooks, where the Clerget divisors are shown for varying values of $P - P'$. These tables are based on the erroneous assumption that the partial sucrose concentration determines the value of the Clerget divisor. They are therefore applicable only to pure sucrose, or within certain limits to products of high sucrose purity. Still other tables which have been widely reprinted in textbooks give the Clerget divisors on the basis of the saccharimeter reading of the inverted solution. Although correct for solutions of pure sucrose, these tables are wrong in principle for the reasons already indicated and also because other optically active substances which may be present in the original product besides sucrose, such as glucose or invert sugar, will alter the negative reading.

Concentration affects the determination of sucrose in yet another way. In the original Herzfeld method the solution for the direct polarization contains the normal weight in 100 ml., but the solution for the invert reading has only half that concentration. This procedure is correct for pure sucrose, but if the product analyzed contains other optically active substances whose specific rotation changes with concentration, as invert sugar, they may lead to serious errors. Years ago Gubbe as well at Landolt called attention to this matter. Jackson and Gillis[26] brought it up again, and the work of Zerban and co-

[23] *Louisiana Planter*, 67, 44 (1921).
[24] *J. Assoc. Official Agr. Chem.*, 8, 384 (1925).
[25] See also footnote, p. 266.
[26] *Bur. Standards Sci. Paper* 375, p. 172, 1920.

workers[27] has shown conclusively that the quantity of product in the solution for the direct polarization and in that to be inverted must be the same. The method of the Association of Official Agricultural Chemists, described on p. 407, is based on this procedure.

The correct method of preparing solutions and of calculating the final result is shown in the following example:

Thirteen grams of a 60-Brix sirup containing sucrose and glucose, made up to 100 ml., gave a direct polarization at 23.0° C. of +27.6° V.; $P = 27.6 \times 2 = 55.2$; 13 g. of the same sirup inverted by the Schrefeld method and made up to 100 ml. gave a reading of +3.6 at 23° C.; $P' = 3.6 \times 2 = 7.2$. The dry substance concentration in the original solutions is $60 \times 0.13 = 7.8$ g. in 100 ml. The table on p. 410 shows a Clerget divisor of 132.65 for this concentration at 20° C. The percentage of sucrose in the sirup is then equal to

$$\frac{100(55.2 - 7.2)}{132.65 - 0.5(23 - 20)} = \frac{4800}{131.15} = 36.60 \text{ per cent}$$

Saillard's Modification of the Original Clerget Method. In France the original Clerget procedure is still generally employed. Since the operating conditions are quite different from those in the Herzfeld method, the concentration factor in the Clerget formula is naturally different also. Saillard[28] has determined the concentration factor for the following inversion procedure. The French normal weight (16.269 g.) is dissolved to 100 ml., and the polarization read in the French instrument (P). Another normal solution is prepared in a 100–110-ml. flask, and the 110-ml. volume is completed by the addition of hydrochloric acid of 22° Baumé (35.21 per cent). The solution is gradually heated from 20° to 70° C. in a total time of 11 minutes, and the flask is then plunged into water at 20° C. The invert reading is multiplied by 1.1, giving P'. The percentage of sucrose is calculated by the formula

$$S = \frac{100\ (P - P')}{134.0 + 0.0928\ (g - 16.269) - 0.5\ (t - 20)}$$

where g is grams dry substance in 100 ml. of solution before addition of the acid. The basic Clerget divisor was found to be the same as that of Clerget.

Walker's Inversion Method.[29] This method was designed to avoid the destructive effect on fructose of prolonged heating at a high temperature. The Association of Hawaiian Sugar Technologists has

[27] *J. Assoc. Official Agr. Chem.*, **8**, 384 (1925).
[28] *Compt. rend.*, **181**, 139 (1925).
[29] *J. Ind. Eng. Chem.*, **9**, 490 (1917).

adopted this method for preparing the inverted solution in the following form:[30]

Place 75 ml. of the clarified and filtered solution used for the direct polarization in a 100-ml. flask, and heat in a water bath to 65° C. For analysis of sugar, take 50 ml. and add 25 ml. of water. Remove from bath and immediately add 10 ml. of hydrochloric acid of 24.85° Brix. Allow to stand for 30 minutes (longer standing does not affect results), bring to room temperature, make up to the 100-ml. mark, shake, filter if necessary, and polarize in a water-jacketed tube, taking the temperature at time of observation.

The percentage of sucrose is calculated by the formula

$$S = \frac{100\ (P - P')}{133.2 + 0.0676\ (g - 13) - 0.5\ (t - 20)}$$

It is to be noted that the concentration of dry substance in the solution for the direct polarization and in that to be inverted is not the same, and that considerable errors may result from this.

Jackson and Gillis recommend[31] Walker's inversion method, but with elimination of the error just mentioned. After the solution has been acidified at 65° C. it is allowed to stand for 15 minutes and made up to the mark at 20° C. The basic Clerget divisor at 20° C. was found to be 133.25.

Several investigators, especially in the temperate zone, have reported incomplete inversion by Walker's procedure when it is applied to low-purity products or even to raw sugars. The results are evidently influenced by the rate of heating as well as by the rate of cooling, both of which depend on the room temperature. In the directions given for the Hawaiian modification it is recommended that 1 to 2 ml. of hydrochloric acid of 24.85° Brix be added to the solution before heating, to neutralize any excess of lead subacetate present. This addition of acid also displaces the weak acids of the salts present in low-purity products so that the 10 ml. of acid added later may have their full effect. Nevertheless, the method should be used with caution, and the chemist should always satisfy himself that complete inversion is obtained.

Inversion at Ordinary Temperature. The dangers of too high or too prolonged heating in the Clerget determination may be avoided by inverting at the ordinary laboratory temperature. The time necessary to invert a half-normal weight (13 g.) of sucrose in 100 ml. of

[30] Methods of Chemical Control, 2nd ed., p. 41, 1931.
[31] *Bur. Standards Sci. Paper* 375, pp. 129, 181, 1920.

solution employing hydrochloric acid of 1.18 sp. gr. was found by Hammerschmidt[32] to be as follows:

Temperature	5 ml. HCl	10 ml. HCl
° C.	hours	hours
10	225	94
15	101	44
20	47	20
25	23	10
30	11.6	5

Jackson and Gillis[31] have made a careful study of the inversion velocity when 50 ml. of sucrose solution, 20 ml. of water, and 10 ml. of hydrochloric acid, *d* 1.1029 at 20°/4° C., are allowed to stand at various temperatures. Their results are assembled in Table LXV.

TABLE LXV

INVERSION VELOCITY AT VARIOUS TEMPERATURES

Temperature, ° C.	Time Required for 99.99 Per Cent Inversion
20	30.8 hours
25	14.6 hours
30	7.1 hours
35	3.5 hours
40	106 minutes
50	29.3 minutes
60	8.7 minutes

They recommend that the solutions to be inverted be allowed to stand for the time specified in the table. The solution is then quickly cooled to 20° C., made up to volume, and polarized.

The Association of Official Agricultural Chemists recommends that the solutions be allowed to stand for 24 hours at a temperature not below 20° C., or for 10 hours if the temperature is above 25° C. It is safer to let low-purity products stand a few hours longer to insure complete inversion.

As destruction of invert sugar is largely avoided by operating at room temperature the Clerget divisor is slightly higher than when the inversion is carried out at 67° to 70° C. Jackson and Gillis found that with inversion at 60° C. or below, for the time specified in Table

[32] *Z. Ver. deut. Zucker-Ind.*, 40, 465 (1890).

LXV, the basic divisor at 20° C. is 133.25.[32a] When working at room temperature, Schrefeld found 133.2, and Zerban and coworkers 133.18. The average of these is 133.21, or in a round figure 133.2. This last value has been adopted by the Association of Official Agricultural Chemists for inversion at room temperature by Schrefeld's procedure.

Effect of Amount of Acid on the Clerget Factor. The effect of varying the quantity of hydrochloric acid used for inversion upon the Clerget factor was studied by Hammerschmidt,[33] who obtained the following invert readings at 20° C. for a normal weight of pure sucrose, using 5, 10, 15, and 20 ml. of hydrochloric acid per 100 ml.

	5 ml.	10 ml.	15 ml.	20 ml.
Reading of normal weight (degrees Ventzke)	−34.00	−35.04	−35.95	−36.80
Reading of ½ normal weight × 2 (degrees Ventzke)	−33.00	−34.12	−35.15	−36.03

It will be noted that there is a pronounced but diminishing increase in the invert reading with the addition of each 5 ml. of acid.

Steuerwald's Method of Inversion at Room Temperature. When inversions are made at room temperature, with the usual quantity of hydrochloric acid, the time required is too long for purposes of factory control, and the advantage of avoiding destruction of invert sugar is largely lost. Steuerwald[34] has overcome this objection by increasing the quantity of acid used for inversion from 10 ml. to 30 ml. of acid of sp. gr. 1.10. The mixture is allowed to stand for 3 hours at temperatures between 20 and 25° C., or for 2 hours above 25° C. The solution is then made up to 100 ml., mixed, and polarized with the usual precautions. Steuerwald gives 135.54 as the basic Clerget divisor at 20° C. for this method of inversion. The concentration factor was found to be 0.0683 instead of 0.0676. The complete Clerget formula is therefore

$$S = \frac{100\,(P - P')}{135.54 + 0.0683\,(g - 13) - 0.5\,(t - 20)}$$

Browne's Modification of the Clerget Method at Room Temperature. In order to avoid doubling any error in observation produced by the dilution of the inverted solution from 50 to 100 ml., Browne[35] has

[32a] In a later investigation, Jackson and McDonald observed that the divisor varies even when the inversion is carried out at temperatures below 60° C. With inversion at 60° they found 133.18; at 49° and at 35°, 133.25; and at 25°, 133.29. They ascribe this to the destructive effect of the acid on the fructofuranose formed initially in the inversion of sucrose (*J. Assoc. Official Agr. Chem.* 22, 580).

[33] *Z. Ver. deut. Zucker-Ind.*, 40, 465 (1890).

[34] *Arch. Suikerind.*, 21, 831 (1913); *Intern. Sugar J.*, 15, 489 (1913).

[35] *J. Assoc. Official Agr. Chem.*, 2, 135 (1918/19).

recommended a return to the original procedure of Clerget in which the volume of the inverted solution is increased by only one-tenth. But in recommending this he advocated the correction of a serious error in the old Clerget procedure due to the diminution of volume during inversion, which is produced by the following causes:

(1) The contraction in volume which all sucrose solutions undergo during inversion and which for 13 g. of sucrose in 55 ml. is about 0.25 ml.

(2) The elevation in temperature produced by the addition of the hydrochloric acid. This elevation, for 5 ml. of concentrated hydrochloric acid to 50 ml. of sugar solution, is about 3° C.; the cooling of the solution from 23° C. at the beginning to 20° C. at the end of the inversion produces a further slight contraction.

(3) The evaporation of water from the neck of the flask during inversion, the amount of such evaporation depending upon the diameter of the neck of the flask, and the time and temperature of inversion.

The combined influence of these three factors causes the volume of the 55 ml. of solution at the end of inversion to be about one-third of a milliliter too small for the half-normal weight of 13 g. In order to correct for this diminution in volume and to prevent decomposition of fructose the following method of inversion at room temperature is employed: 50 ml. of the solution used for the direct polarization is measured into a 50–55-ml. flask and 5 ml. of concentrated hydrochloric acid (1.19 sp. gr.) is added. After the flask has stood overnight at room temperature, which should not be below 20° C., the volume is completed to exactly 55 ml., after the walls of the flask are gently tapped to detach any air bubbles. The solution is then mixed and polarized with the usual precautions. The invert reading is corrected by adding one-tenth and the sucrose calculated by the formula

$$S = \frac{100\,(P - P')}{133.15 + 0.0673g - 0.5(t - 20)}$$

where g equals grams dry substance in 100 ml. of the original solution used for the direct polarization. The basic divisor for 20° C. and for 26 g. of dry substance in 100 ml., or 13 g. in 50 ml., is 134.9. The figure 133.15 in the above formula is for zero concentration of sucrose. For the French normal weight of 16.269 g. the divisor is $134.25 - 0.5\,(t - 20)$.

Saillard also made inversions at room temperature by his modification of Clerget's original procedure (p. 412), and found the same basic factor 134.0 at 20° C. for the French normal weight as he did for his method of heating from 20° to 70° C. in 11 minutes.

MODIFICATIONS OF THE DOUBLE POLARIZATION METHOD FOR IMPURE SUGAR PRODUCTS

Effect of Fructose on the Clerget Factor. Owing to the influence of hydrochloric acid upon the polarization of fructose a Clerget formula based upon the inversion of pure sucrose by means of this acid is not absolutely correct when applied to the analysis of impure products containing invert sugar, since the specific rotation of fructose is different in the neutral and acid solutions before and after inversion. A considerable error is introduced, in fact, if the Clerget formula established for pure sucrose is employed in the examination of molasses, honey, jam, jelly, and other materials containing considerable fructose.

Effect of Amino Compounds on the Clerget Factor. The hydrochloric acid used for inversion may also affect the polarization of other ingredients than fructose. Low-grade molasses, plant extracts, and other sugar-containing materials frequently contain considerable quantities of optically active amino compounds such as asparagine, aspartic acid, glutaminic acid, leucine, and isoleucine, the optical activity of which varies with the alkalinity and acidity of the solution. This may be seen from the following table, which gives the approximate specific rotations of several amino derivatives in alkaline solution, in water, and in hydrochloric acid.

APPROXIMATE VALUE FOR $[\alpha]_D$

	In Presence of NaOH	In Water	In Presence of HCl
Asparagine	− 8	− 6	+34
Aspartic acid	− 9	+ 4	+34
Glutaminic acid	−68	+10	+20
Leucine	+ 7	....	+17
Isoleucine	+11	+10	+37

The influence of such variations upon the Clerget calculation is illustrated in the work of Andrlík and Staněk,[36] who showed that a 1 per cent solution of glutaminic acid gave a reading of —1.45° V. in the presence of lead subacetate, —0.35° V. in water alone, and +1.77° V. in dilute hydrochloric acid. In the case of an osmose water from a beet-sugar factory the direct polarization was 14.75° V. in alkaline, 14.85° V. in neutral, and 15.80° V. in acid solution. Ehrlich[37] had previously also called attention to the large errors in the Clerget

[36] *Z. Zuckerind. Böhmen,* 31, 417 (1906/07).
[37] *Z. Ver. deut. Zucker-Ind.,* 53, 809 (1903).

method due to the presence of amino compounds. Eisenschimmel[38] found that a 0.1 per cent solution of glutamine reads +0.08° V. before inversion, and +0.25° after inversion with hydrochloric acid at 69° C.

When reducing sugars and amino compounds are present simultaneously in alkaline solution they tend to form complexes and condensation products of varying rotation, as has been pointed out by Englis and Dykins.[39] The change in rotation increases with the *p*H, with the ratio of amino compound to sugar, with the temperature, and with the time. Neutralization does not always restore the original rotation. This phenomenon is of importance when alkaline substances, like sodium carbonate, are used for deleading and for completing mutarotation, and may lead to appreciable error in sucrose determinations in plant juices high in nitrogen and low in sugars.

It is evident that to overcome the variations in specific rotation of fructose, amino compounds, etc., which occur in the presence and absence of hydrochloric acid, the original method of Clerget must be considerably modified in the case of impure products. Several such modifications of the method have, in fact, been devised, and these for convenience may be grouped into two general classes. I. Clerget modifications which attempt to equalize the conditions before and after inversion with acids. II. Clerget modifications which employ an inverting agent free from the objections to hydrochloric acid.

Among the modifications of Class I may be mentioned the following:

Saillard's Method of Neutral Double Polarization.[40] In this method the solution which has been inverted with hydrochloric acid is, after cooling, carefully neutralized with sodium hydroxide, an excess of alkali being avoided. In order that the direct polarization may be made under similar conditions, Saillard recommends that sodium chloride, equivalent to the amount present after neutralizing the acid in the inverted solution, be added to the solution for the direct reading before making up to volume.

Later Saillard[41] found that the salts originally present in beet molasses have a specific effect on the Clerget divisor, and also that 5 ml. of acid is usually not sufficient for complete inversion at the temperature and during the time required for the inversion of pure sucrose. Saillard therefore recommends the determination of the sulfated ash in the product. For both the direct and the invert polarization a solution containing 13 (or 16.269) g. of the product in 50 ml. is prepared,

[38] *Z. Zuckerind. čechoslovak. Rep.*, **51**, 337 (1926/27).
[39] *Ind. Eng. Chem., Anal. Ed.*, **3**, 17 (1931).
[40] *Proc. 8th Intern. Congr. Appl. Chem.*, **25**, 541 (1913).
[41] *Z. Ver. deut. Zucker-Ind.*, **64**, 841 (1914).

and the volume is in each case completed to 100 ml. after addition of the chemicals. An amount of concentrated hydrochloric acid equivalent to the salts present in the product is added to the solution for the invert polarization, besides the 5 ml. used for the inversion itself. The acid is neutralized with sodium hydroxide before the reading is taken. To the solution for the direct polarization is added a quantity of sodium chloride equivalent to the total amount of acid used in the inverted solution.

To establish the Clerget divisor, a sucrose solution is prepared of such concentration that the direct polarization is about the same as that of the solution of the product. For the inversion the usual 5 ml. of hydrochloric acid is added, and after inversion is complete, a further quantity of acid equal to the excess acid used in the analysis of the product plus that equivalent to the salts in the solution of the product. The acid is neutralized with sodium hydroxide before the reading is taken. To the solution for the direct polarization is added a quantity of sodium chloride equivalent to the total amount of acid used in the inverted solution. The Clerget divisor is then calculated from the direct and invert readings of the sucrose-salt solution and the direct polarization of the pure sucrose solution.

This method has a sound theoretical foundation, but it is too cumbersome for practical purposes as it requires the preparation and polarization of five different solutions. Furthermore, it is not applicable to low-purity cane products, which always contain reversion products that are hydrolyzed by acid.

Jackson and Gillis's Methods with Compensating Quantities of Reagents. An extensive study of acid hydrolysis methods of double polarization has been made by Jackson and Gillis.[42] They observed that the slightest excess of sodium hydroxide, the neutralizing reagent recommended by Saillard, destroys fructose very rapidly. Even an excess within the limits of error of ordinary titration has this effect. They therefore adopted ammonia, which they found to be free from that objection. The titration is carried out with methyl orange as indicator, in a separate portion of the acid used for inversion.

The various inversion procedures recommended by Jackson and Gillis have already been described on pp. 408, 413, and 414. The concentration of the solution to be inverted is always the same as that of the solution for the direct reading.

Jackson and Gillis recommend four different methods of double polarization, all based on the essential principle that " the rotation of all substances except sucrose must be kept constant in the two polariza-

[42] *Bur. Standards Sci. Paper* 375, 1920.

tions." Method I is a modification of the Herzfeld procedure and has been described on p. 408. It is applicable only to pure sucrose and to mixtures which are free from impurities whose rotation is affected by hydrochloric acid. The basic Clerget divisor is 133.25 at 20° C.

Method II is strictly a method of neutral double polarization and, according to Jackson and Gillis, is applicable to all products of whatever nature. The solution, inverted with 10 ml. of hydrochloric acid of density 1.1029, is quickly cooled, and the free hydrochloric acid is neutralized with the predetermined quantity of ammonia which is slowly added from a burette with constant stirring. To compensate for the effect of the ammonium chloride formed, 3.392 g. of ammonium chloride, or 15 ml. of a solution containing 226 g. of the salt per liter, is added to the solution for the direct polarization before completing the volume. The Clerget constant for this procedure is given as 133.34 at 20° C.

Method III has been devised for products which are virtually free from invert sugar but do contain amino compounds whose rotation changes with hydrogen-ion concentration; beet products generally fall in this class. The direct polarization is carried out without any addition of reagents, while the inverted solution is neutralized with ammonia as described under method II. The Clerget constant was found to be 133.91 at 20° C.

Method IV may be used for those products which contain invert sugar but are free from amino compounds whose rotation depends on the reaction. The inverted solution is read in the presence of the free hydrochloric acid, but the effect of the acid is counterbalanced by adding to the solution for the direct reading, before making up to volume, 2.315 g. of sodium chloride, or an equivalent amount of this salt in solution. The Clerget constant is given as 132.63 at 20° C. This method has been adopted by the United States Customs Service and also in the "Book of Methods" of the Sugar Club of Cuba, for the analysis of cane products. The effect of the amino compounds occurring in cane products is thus considered to be negligible.

The basic constants given above for these methods must be corrected for concentration by adding 0.0676 $(g - 13)$, where g is again the grams of dry substance in 100 ml. of solution. Jackson and Gillis[43] recommend a temperature coefficient of $-0.53\ (t - 20)$, instead of the commonly used $-0.5\ (t - 20)$.

Browne[44] has called attention to the fact that, while soluble salts do depress the polarization of sucrose, as shown on p. 276, this depres-

[43] *Bur. Standards Sci. Paper* 375, p. 184, 1920.

[44] *Louisiana Planter*, 66, 109 (1921).

sion is not constant for all concentrations of sucrose unless the ratio between salt and water in the 100 ml. of solution is constant, a condition which is not realized in analytical practice. This may be seen from the following results:

Sucrose in 100 ml.	Salt in 100 ml.	Water in 100 ml.	Polarization in Terms of 26 g. °V.
26 g.	5 g.	82.0 g.	98.58
13 g.	5 g.	90.1 g.	98.83

When less sucrose is taken, more water is needed to complete the volume and the depressing action of the salt upon the rotation is correspondingly diminished. As the varying amounts of sucrose and invert sugar of complex mixtures do not have constant rotations in the presence of a fixed amount of salt in 100 ml., owing to the varying water content of the solution, the methods of making direct and invert polarizations in solutions containing the same amount of soluble salts cannot be regarded as exact.

Deerr's Method of Neutral Double Polarization.[45] This method was devised for a dual purpose: first to overcome the objections to the use of lead salts for clarification, and second to make the direct and invert polarizations in a solution neutral in reaction, without any added salts remaining in solution. The method is based on the complete precipitation of barium sulfate and aluminum hydroxide when equivalent solutions of barium hydroxide and aluminum sulfate are mixed, and of barium sulfate alone when the equivalent quantity of barium hydroxide is added to sulfuric acid. The original method of Deerr has been modified by Coates and Shen,[46] and their procedure has been adopted by the Sugar Technologists' Association of India in the following form:[47]

The following reagents are required:

A. A 0.5 *N* solution of barium hydroxide, prepared by dissolving 76 g. of $Ba(OH)_2 \cdot 8\ H_2O$ in 1 liter of water and filtering.

B. An aqueous solution containing 190 g. crystallized aluminum sulfate $Al_2(SO_4)_3 \cdot 18\ H_2O$ in 1 liter total volume.

C. A solution of 129 ml. sulfuric acid of sp. gr. 1.8354 diluted with water to 500 ml.

D. Rosolic acid indicator, made by dissolving 0.5 g. of the acid in 50 ml. of water and 50 ml. of alcohol.

[45] *Intern. Sugar J.*, 17, 179 (1915).
[46] *Ind. Eng. Chem.*, 20, 70 (1928).
[47] "Methods of Chemical Control," pp. 110–112, 1936.

Solution A is titrated against B, and the concentration of B is adjusted so that 25 ml. of A exactly neutralizes 15 ml. of B. Solution A is also titrated against C and the value noted.

For the direct polarization, 50 ml. of a normal or half-normal solution is introduced into a 200-ml. flask, 25 ml. of solution A is added, and, after mixing, 15 ml. of B is run in during constant agitation. The volume is completed, the solution filtered, and the filtrate is polarized, preferably in a 400-mm. tube. A deduction of 0.3 per cent of the reading is applied to compensate for the volume of the precipitate produced in clarification.

Another 50-ml. portion is pipetted into a 200-ml. flask, and 5 ml. of solution C is added. The flask is then placed in a water bath heated to 73° C., and a thermometer is inserted in the flask. The temperature of the solution should reach 68° C. in 3 minutes; it is then kept at this temperature for 7 minutes longer. The flask is rotated from time to time during the heating to insure a uniform temperature. The flask is then rapidly cooled to room temperature, 15 ml. of solution B and 3 drops of rosolic acid indicator are added, and solution A is run in from a burette until the solution is distinctly pink. One drop of solution C is added to bring the solution back to neutrality, the volume is completed to the mark, the solution filtered and polarized. A correction of 0.89 per cent of the reading is deducted to compensate for the volume of the precipitate.

Coates and Shen found the following Clerget divisors for this method, at 20° C.: 131.7 for a half-normal solution, 130.9 for a quarter-normal, and 130.6 for an eighth-normal. The Clerget formula for this method is therefore:

$$S = \frac{100\ (P - P')}{131.7 + 0.1187\ (g - 13) - 0.5\ (t - 20)}$$

The divisor 131.7 is 0.4 lower than the sum of the direct and invert polarizations of a half-normal weight of sucrose. It is evident that a considerable amount of fructose is destroyed at the temperature of 68° C.

Deerr's method is used quite generally in India but has not found favor elsewhere. It is very doubtful whether the volume corrections of 0.3 and 0.89 per cent, established with pure sucrose, apply also to low-purity products. The very fact that the clarifying agents markedly decolorize the solutions shows that the precipitate absorbs non-sugars, and the volume of the precipitate must be greater for low-purity products than for pure sucrose. Difficulties are often encountered in filtering off the precipitates, which have a tendency to run

through the filter. The half-normal solution of barium hydroxide will crystallize in the temperate zone during the winter, and it is necessary to employ more dilute solutions of reagents A, B, and C.

Direct Polarization in Presence of Hydrochloric Acid and Urea. This modification, due to Andrlík and Staněk,[48] is based upon the retarding influence which urea (or betaine) exercises upon the inversion of sucrose with hydrochloric acid in the cold. Fifty milliliters of the solution for the direct polarization are made up to 100 ml. with a solution containing 5 g. urea and 5 ml. strong hydrochloric acid per 50 ml. of reagent. After mixing, the solution is filtered and polarized as quickly as possible. It is claimed by the authors of the method that a sufficient interval (7 to 10 minutes) elapses before inversion is noticeable to make the direct polarization. Though this claim may be true for certain classes of products, it certainly is not true with substances rich in sucrose. The following experiment shows a comparison of the rate of inversion of 13 g. of sucrose at 20° C. in the presence of 5 ml. of strong hydrochloric acid and in the presence of 5 ml. of strong hydrochloric acid plus 5 g. urea in 100 ml. of solution.

TABLE LXVI

INFLUENCE OF UREA UPON THE RATE OF INVERSION OF SUCROSE

Time	Inversion with 5 ml. HCl		Inversion with 5 ml. HCl + 5 g. Urea	
	Reading, °V.	Velocity Constant k	Reading, °V.	Velocity Constant k
0 min.	+49.9	.....	+49.9	.....
2 min.	49.4	0.0016	49.6	0.0009
5 min.	48.9	0.0013	49.4	0.0007
7 min.	48.6	0.0012	49.3	0.0005
10 min.	48.0	0.0012	49.1	0.0005
30 min.	44.3	0.0013	47.2	0.0006
60 min.	39.7	0.0012	44.8	0.0006
120 min.	31.4	0.0012	40.1	0.0006
180 min.	24.7	0.0012	35.8	0.0006
2 days	−16.5	.....	−17.2	.....
4 days	−16.5	.....	−21.3	.....
Average	.....	0.00128	.....	0.00063

Taking the reading before inversion as +49.9 and the reading at completion of inversion as −16.5 it is seen that the velocity of inver-

[48] *Z. Zuckerind. Böhmen*, 31, 417 (1906/07).

sion $\left(k = \frac{1}{t} \log \frac{a}{a - x}\right.$, see p. 402$\left.\right)$ is diminished one-half by the addition of 5 g. urea. There is no suspension of the inversion at the beginning, there being a decrease of 0.3 in the reading at the end of 2 minutes, and of 0.5 after 5 minutes. Under such circumstances it is impossible to take the true direct polarization.

A second objection to the Andrlík-Staněk modification is that the method cannot be used when reducing sugars are present owing to the change which the urea causes in their specific rotation. The extent of this change can be seen from the following experiments upon solutions of fructose, glucose, and invert sugar. The same volume of sugar solution was taken in each case and, after addition of substance, was completed to 100 ml. The readings were taken immediately except as otherwise stated.

	Fructose	Glucose	Invert Sugar
Volume completed with water alone	−26.2° V.	+56.5° V.	−10.2° V.
Volume completed with water + 5 g. urea	−27.0	+56.1	−10.6
Volume completed with water + 5 ml. HCl	−26.9	+56.7	−10.5
Volume completed with water + 5 g. urea + 5 ml. HCl	−27.3	+56.5	−10.7
Volume completed with water + 5 g. urea + 5 ml. HCl after 2 days	−27.3	+48.0	−11.9

It is seen that the 5 g. urea + 5 ml. hydrochloric acid produce a different rotation from the 5 ml. hydrochloric acid alone, this difference being greater for fructose. On long standing, glucose in the presence of hydrochloric acid and urea shows a loss in rotation owing to the formation of glucose ureide ($[\alpha]_D = -23.5$). This explains the high levorotation of invert sugar solutions prepared in presence of urea. (See Table LXVI.)

The Andrlík-Staněk method is a dangerous one, for it may introduce greater errors than those which it was designed to correct. The process, notwithstanding several favorable notices in the literature, is not to be generally recommended.

Direct and Invert Polarizations in the Presence of Hydrochloric Acid and Potassium Citrate or Potassium Acetate. Staněk[49] tried to overcome some of the objections to the preceding method by inverting with hydrochloric acid at high temperature as usual and then adding

[49] *Z. Zuckerind. Böhmen*, 38, 429 (1913/14).

an equivalent quantity of potassium citrate solution; in the solution for the direct polarization the same quantities of hydrochloric acid and potassium citrate were also added. Staněk found that the citric acid displaced by the hydrochloric acid from its potassium salt has such a small inverting effect at room temperature that it is not necessary to operate as rapidly as with the urea method. No lead salts are used for clarification in this method, but the solutions are decolorized by oxidation with bromine water. Babinski and Ablamovitch[50] substituted the cheaper sodium acetate for the potassium citrate recommended by Staněk. Schlemmer[51] further modified the method by using potassium bromide and chloramine-T (the sodium salt of *N*-chloro-para-toluenesulfonamide, sold under the trade name of Activin) instead of the bromine water which is objectionable because of its irritating odor. A solution containing 400 g. of sodium acetate and 50 g. potassium bromide per liter is prepared. Fifty milliliters of a normal solution of the sugar product is inverted in a 100-ml. flask by heating with 10 ml. of hydrochloric acid of sp. gr. 1.1029 for 5 minutes at 68.5° C. Then 20 ml. of the acetate-bromide solution is added, and the solution is cooled to room temperature. It is then decolorized with 10 ml. of a 15 per cent aqueous solution of chloramine-T with shaking. The solution is made up to the mark, filtered, and polarized. To another 50-ml. portion of the original solution the same quantities of hydrochloric acid, acetate-bromide solution, and chloramine-T are added directly, the volume is completed to 100 ml., and the solution is well mixed, filtered, and polarized. Only a very slight precipitate is produced by the reagents, not exceeding 0.15 g. in the case of final molasses, and its volume can be neglected or corrected for. The Clerget constant for pure sucrose at 20° C. was found to be 131.98, and it was practically independent of the sucrose concentration; for final molasses the constant is 131.75.

Schlemmer discovered that in all the methods in which acid is used for inversion the *l*-glutiminic and saccharinic acids contained in beet molasses are also hydrolyzed and cause low sucrose results through change in rotation. Only the invertase method avoids this source of error.

Direct and Invert Polarizations in the Presence of Weak Acids. Rolfe and Hoyt[52] observed that mono- or trichloroacetic acid causes no noticeable inversion of sucrose at room temperature within 15 minutes, but that complete inversion can be effected by heating. Trichloroacetic acid was found to have certain disadvantages in its practical

[50] *Gazeta Cukrownicza*, **44**, 10, 147 (1914/15).
[51] *Z. Zuckerind. čechoslovak. Rep.*, **53**, 13 (1928).
[52] *Ind. Eng. Chem.*, **12**, 250 (1920).

application. Rolfe and Hoyt recommend the addition of 3 g. of monochloroacetic acid to 50 ml. of a normal solution and completion of the volume to 100 ml. A part of this solution is read within 15 minutes, and this gives the direct polarization. Another portion of the same solution is heated in a boiling water bath for 30 minutes, or with low-purity products for 60 minutes. The Clerget divisor at 20° C. is given as 131.0 at 20° C. The long heating period at 100° C. makes the method of doubtful value in the analysis of products containing invert sugar.

Soliven[53] has proposed a similar method, in which 10 ml. of phosphoric acid solution, sp. gr. 1.3787, is added to both the solutions for the direct and invert readings, and the inversion is carried out according to the Walker procedure. The solution for the direct reading must be polarized immediately after the addition of the phosphoric acid. Although Soliven reported good checks between the results of his method and those of the invertase method, his procedure cannot be recommended, because of the danger of incipient inversion on the one hand, and of mutarotation effects on the other.

Among the modified methods belonging to Class II, which employ for the Clerget determination inverting agents less open to objections than hydrochloric acid, may be mentioned the following:

Inversion by Means of Organic Acids, without Compensation in the Direct Polarization. Besides the two preceding methods, in which weak acids are added to both solutions for the direct and invert polarizations, a number of others have been proposed where such acids are employed only in the solution to be inverted, on the assumption that they have no pronounced effect on the optical activity of fructose. Weber[54] claimed that in the presence of acetic acid invert sugar has the same rotatory power as in aqueous solution, but this has been disproved by Jackson and Gillis,[55] who found that acetic acid, unlike most other acids and salts, decreases the levorotation of invert sugar. Besides, acetic acid is an unsatisfactory reagent for the Clerget determination on account of its very weak inverting action ($\frac{1}{250}$ that of hydrochloric acid). Tolman[56] has tested the use of citric acid for the Clerget process and found that with 2 g. of this acid in 100 ml. complete inversion of sucrose could be accomplished in 30 minutes at the temperature of boiling water. Under these conditions the Clerget factor for

Intern. Sugar J., 36, 370 (1934).

[54] *J. Am. Chem. Soc.*, 17, 321 (1895).

[55] *Bur. Standards Sci. Paper* 375, p. 156, 1920.

[56] *Bull.* 73, U. S. Bur. Chem., p. 69.

the normal weight of sucrose was 141.95 and for the half-normal weight 141.49. Tolman noted, however, that the presence of soluble acetates greatly retarded the inverting action of citric acid and that this acid was consequently of no value as an inverting agent with products which required previous clarification with lead subacetate. This same objection would apply to many other organic acids. Another serious objection, as with hydrochloric acid, against the use of organic acids as inverting agents is the difference in optical activity of contaminating amino compounds in the solutions used for direct and invert polarization — asparagine, for example, being levorotatory in aqueous solution, but dextrorotatory in the presence of strong acetic acid.

Oxalic acid[57] has also been recommended as an inverting agent, 2 g. of the acid being used for 100 ml. of solution. This acid has a much stronger inverting power than either acetic or citric acid, but is open to the same objections previously stated.

The employment of organic acids as inverting agents in the examination of impure sugar products has not been found to be satisfactory.

Inversion by Means of Invertase. The employment of yeast as an inverting agent in the Clerget determination of sucrose was first indicated by Kjeldahl[58] in 1881. O'Sullivan and Tompson,[59] in 1891, and Ling and Baker[60] in 1898, extended the use of the method and later Ogilvie applied it to the analysis of sugar-factory products. The yeast method of O'Sullivan and Tompson, as modified by Ogilvie,[61] is as follows:

Four times the normal sugar weight of the sample are transferred to a standardized 200-ml. flask, defecated with the minimum amount of basic lead-acetate solution (sp. gr., 1.26), a little alumina cream added, then the liquid adjusted to bulk at standard temperature, well shaken, and filtered; 100 ml. of the filtrate is measured by a standard pipette into a small beaker, sulfur dioxide passed in from a siphon of the liquefied gas till a faint smell is perceptible (all the lead thus being indicated to be precipitated), then the liquid transferred to a 200-ml. flask, made up to the mark, and well mixed. Now sufficient calcium carbonate (dried) in fine powder to neutralize the excess of acidity, and a little recently ignited kieselguhr (to promote filtration) are added, after which filtration follows. In this way a normal solution is obtained, which is sufficiently clarified to give a distinct polarimetric reading,

[57] Kulisch, *Z. angew. Chem.*, **1897**, 45.
[58] *Compt. rend. Lab. Carlsberg*, **1**, 192 (1881).
[59] *J. Chem. Soc. Trans.*, **59**, 46 (1891).
[60] *J. Soc. Chem. Ind.*, **17**, 111 (1898).
[61] *Intern. Sugar J.*, **13**, 145 (1911).

is free from lead and excess of acidity, and is therefore well suited for the invertase inversion.

Fifty milliliters of the solution, prepared in the manner just described, contained in a 100-ml. flask, is raised in a constant-temperature bath to between 50° and 55° C., after which 0.5 g. of washed brewery yeast and 2 drops of acetic acid are added and the temperature maintained as near 55° C. as possible for $4\frac{1}{2}$ to 5 hours. At the end of this time the liquid is cooled, and a little alumina cream or kieselguhr added to assist filtration, and made up to bulk at standard temperature. The clear filtrate is then polarized in a lateral-branched water-jacketed tube at exactly 20.0° C.

The Clerget factor determined by Ogilvie for the above process from experiments upon pure sucrose is 141.6, at a concentration of 6.5 g. in 100 ml. of solution, and 0° C.

Instead of employing yeast, a solution of invertase prepared therefrom may be used to advantage. Hudson[62] developed a method upon this principle. A stock solution of invertase is prepared as follows:

Break up 5 pounds of pressed yeast, which may be either baker's or brewer's yeast, add 30 ml. of chloroform to it in a closed flask and allow it to stand at room temperature (20° C.) overnight. By the morning, the solid mass will have become fluid and it should then be filtered through filter paper, allowing several hours for draining. To the filtrate add neutral lead acetate until no further precipitate forms and again filter. Precipitate the excess of lead from the filtrate with potassium oxalate and filter. To this filtrate add 25 ml. of toluene and dialyze the mixture in a pig's bladder for 2 or 3 days against running tap water. The dialyzed solution is colorless, perfectly clear after filtration, neutral to litmus, has a solid content of about one-half of 1 per cent, an ash content of a few hundredths of 1 per cent, will keep indefinitely in an ice box if a little toluene is kept on its surface to prevent the growth of microorganisms, and is exceedingly active in inverting cane sugar. The invertase solution does not reduce Fehling's solution.

The solution of invertase prepared by this method gives a dextrorotation of 1° V. in a 400-mm. tube.

The Clerget method with invertase is thus described by Hudson:

Dissolve 26 g. of the substance to be analyzed for cane sugar in water, clarify with the usual substances (neutral or basic lead acetate or alumina cream or kaolin), and make up to 100 ml. volume at 20° C. Filter and read the polarization of the filtrate in a 200-mm. tube. Remove the excess of lead from the filtrate, if lead has been used as clarifying agent, with sodium carbonate or potassium oxalate, and filter. To 50 ml. of the filtrate add acetic acid by drops until the reaction is acid to litmus, add 5 ml. of the stock invertase solution, and make up the volume to 100 ml. Add a few drops of toluene to the

[62] *J. Ind. Eng. Chem.*, **2**, 143 (1910).

solution to prevent the growth of microorganisms, shaking so as to saturate, and allow to stand at any temperature between 20° and 40° C. overnight. Under usual conditions about 6 hours' time is required to accomplish complete hydrolysis.

When the inversion is finished, the solution is read at 20° C. and the invert reading calculated to the normal weight of substance. The Clerget factor for the above method as determined by Hudson from experiments upon a solution containing 7 g. of pure sucrose in 100 ml. is 141.7, at 0° C.

In order to eliminate the danger of incomplete inversion, Browne[63] has recommended the following modification of the invertase method: Dissolve 26 g. of substance in water, clarify, make up to 100 ml., and take the direct polarization P at the temperature T. Remove the excess of lead from the filtrate, if lead has been used as a clarifying agent, with powdered anhydrous potassium oxalate and filter. To 50 ml. of the filtrate in a 100-ml. flask add acetic acid by drops until the reaction is acid to litmus paper, add 10 ml. of Hudson's invertase solution and let stand in a warm place (about 40° C.) overnight. Cool to room temperature, make up to 100 ml., and polarize in a 200-mm. tube. Allow the solution to remain in the tube for an hour, and repeat the polarization. If there is no change from the previous reading, the inversion is complete, when the reading and temperature (t) of the solution are carefully noted. The reading is corrected for the optical activity of the invertase solution (10 ml. made up to 100 ml.) and then multiplied by 2, which gives P'. The percentage of sucrose is then calculated by the formula

$$S = \frac{100\ (P - P')}{132 + 0.0660\ (g - 13)}$$

if both readings are taken at 20° C.

If both readings are taken at the same temperature, but not at exactly 20° C., the divisor is corrected by the expression $-0.53\,(t - 20)$; if the direct polarization is read at T° C., and the invert polarization at t° C., the total correction is $-0.5\,(t - 20) - 0.03\,(T - 20)$. But these corrections are valid only for pure sucrose, and not when invert sugar is present in the product to be analyzed.

The application of the above formula to the determination of sucrose in the presence of various amounts of invert sugar is shown by the analyses of the following mixtures:

[63] *J. Assoc. Official Agr. Chem.*, 2, 139 (1916).

Sucrose Taken	Invert Sugar Taken	Direct Polarization	Corrected Invert Polarization 20° C.	Calculated Sucrose
per cent	per cent	*P*	*P'*	*S*
99.80	0.00	+99.80	−31.83	99.72
95.96	0.69	+95.85	−30.83	96.00
90.20	1.65	+89.75	−29.33	90.27
86.37	2.58	+85.60	−28.23	86.31
80.61	3.54	+79.50	−26.73	80.62
49.90	9.38	+47.00	−18.63	49.88
30.71	9.38	+27.90	−12.43	30.69

The inverting power of the stock invertase solution should be carefully determined from time to time by experiments upon pure sucrose, and with any decrease in activity the quantity of reagent used for inversion must be correspondingly increased. The time of inversion can be shortened considerably by conducting the inversion at a temperature of about 55° C. To determine whether or not inversion is complete the closed flask or tube of solution may be warmed again to 55° C. for an hour and then, after cooling to 20° C., reread. If no change in polarization is noted, the inversion is complete.

Preparation of Highly Active Invertase. Invertase prepared by Hudson's original method has a rather low inverting power; the inversion requires considerable time even at higher temperatures, and there is always some uncertainty about the completion of the inversion. For this reason invertase was little used except in research until procedures for preparing a more active enzyme were devised. It was found that a purer invertase is obtained if the autolysis of the yeast is interrupted after a few hours, the yeast filtered, and the filtrate, which contains little enzyme, discarded. The yeast is again mixed with water and chloroform or toluene, and the autolysis is permitted to proceed for several days to completion. The invertase is further purified and concentrated either by adsorption or by ultrafiltration.

Adsorption Method. The principle of the adsorption method, as developed particularly by Willstätter and his school,[64] is illustrated by the following example. The invertase is precipitated from the autolysate with alcohol and extracted from the precipitate with dilute acetic acid. Kaolin is added to adsorb the invertase which is then "eluted" by means of a weakly alkaline solution, either ammonia or sodium carbonate or phosphate. The solution is dialyzed and then adjusted to

[64] *Ann.* **425**, 1 (1921); **427**, 111 (1922); *Z. Physiol. Chem.*, **123**, 1 (1922); **133**, 193 (1924); **142**, 257 (1925); **146**, 158 (1925); *Proc. Royal Soc. London*, **111B**, 282 (1932).

*p*H 5 to 6. The invertase is next adsorbed from this solution by means of specially prepared alumina, eluted again with a solution of disodium phosphate, and dialyzed once more. The treatment with alumina is repeated several times. This procedure yields a highly active invertase with a time value (see p. 437) of about 0.2 minute, but is very time-consuming. Through further refinements the time value has been reduced to 0.1 minute.

A more rapid method in which bentonite is used as the adsorbent, and which also yields very highly active invertase, has been devised by Adams and Hudson.[65] Add 43 ml. of ether to 430 g. of yeast and allow to stand at 30° C. until the yeast has liquefied. Then add 43 ml. of toluene, 430 ml. of water, and 3.2 g. of sodium carbonate. Four hours after the addition of the ether stir 80 g. of Filter-Cel into the mixture, filter, and discard the filtrate. Add to the residue another 43 ml. of toluene and 430 ml. of water, allow to autolyze at 20° C. for 5 days, and dialyze through Visking sausage casings. Add 265 ml. of dialysate to a mixture of 80 ml. of 0.5 per cent bentonite suspension and 27 ml. of an acetate buffer solution of *p*H 4.1. Centrifuge, wash the residue with 200 ml. of distilled water, and centrifuge again. Elute the adsorbed invertase by gentle shaking with three portions of 40, 30, and 20 ml., respectively, of acetate buffer solution of *p*H 5.7, and dialyze. The invertase solutions thus prepared from bakers' yeast have time values of 0.20 to 0.27 minute and may be kept for several months in a refrigerator without loss of activity. Similar preparations may be made from brewers' yeast. Excellent results have also been obtained with zinc sulfide as the adsorbent, by Richtmyer and Hudson.[66]

Weidenhagen[67] has obtained practically quantitative yields of invertase from yeast autolysates by precipitation with strontium hydroxide, followed by elution with primary ammonium phosphate.

Ultrafiltration Method. The ultrafiltration method developed by Reynolds[68] for the purification and concentration of invertase solutions has been adopted by the Association of Official Agricultural Chemists; it is carried out as follows:[69]

(1) *Crude invertase solution.* Mix yeast with water in the proportion of 10 pounds of compressed bakers' yeast to 5 liters of water. Add 2 liters of toluene and stir thoroughly at frequent intervals during the first 24 hours. Allow to stand for 7 days with occasional stirring and filter by gravity through large

[65] *J. Am. Chem. Soc.*, 60, 982 (1938).
[66] *J. Am. Chem. Soc.*, 60, 983 (1938).
[67] *Z. Ver. deut. Zucker-Ind.*, 86, 473 (1936).
[68] *Ind. Eng. Chem.*, 16, 170, 562 (1924).
[69] " Methods of Analysis, A. O. A. C.," 5th ed., p. 492, 1940.

fluted papers. Mix the residue with 2 liters of water, filter, and combine the filtrates. Purify by adding 15 g. of neutral lead acetate to each liter of extract and filtering on paper after all lead acetate has been dissolved. Complete the purification immediately by dialysis or by washing on the ultrafilter as directed under (3).

(2) *Collodion ultrafilter.* Dissolve 6 g. of soluble (in alcohol and ether mixture) pyroxylin or nitrocellulose such as Astoria's in a mixture of 50 ml. of absolute alcohol and 50 ml. of absolute ether by first adding the alcohol to the cotton, allowing the mixture to stand in a stoppered flask for 10 minutes, adding the ether, and shaking. Allow the solution to stand overnight, pour about 100 ml. into a 2000-ml. cylinder, and coat the entire inside surface of the cylinder with the collodion. Drain, and dry for 10 minutes. Fill with water, let stand 10–15 minutes, pour out the water, and remove the collodion sack. Test for leaks by filling with water. Slit open longitudinally and cut out a circular piece about 7–8 inches in diameter. Cut the bottom from a 2-liter bottle or Erlenmeyer flask and grind the edge smooth. Place it upon the still moist collodion disk, fold the edge of the disk up around the bottle, and cement it thereto with collodion that contains an increased percentage of ether. Place 3 or 4 thicknesses of wet filter paper in an 8-inch Büchner funnel. Place the bottle with the collodion membrane upon the filter paper. Pour melted vaseline, to the depth of an inch, between the bottle and inside of the funnel. Provide the bottle with a small mechanical stirring device.

(3) *Washing and concentration of invertase solution by ultrafiltration.* Filter 4 liters of the partially purified solution through the ultrafilter, stirring continuously, until about 1 liter remains. Wash with distilled water introduced by means of a constant-level device until the filtrate is colorless, 3 or 4 liters of wash water being required. During the entire process the invertase solution should be preserved with toluene.

A diagram of the ultrafiltration apparatus is shown in Fig. 204. Ready-made membranes of Cellophane, or Bechhold's ultrafilters, have not been tried as yet in the preparation of invertase solutions.

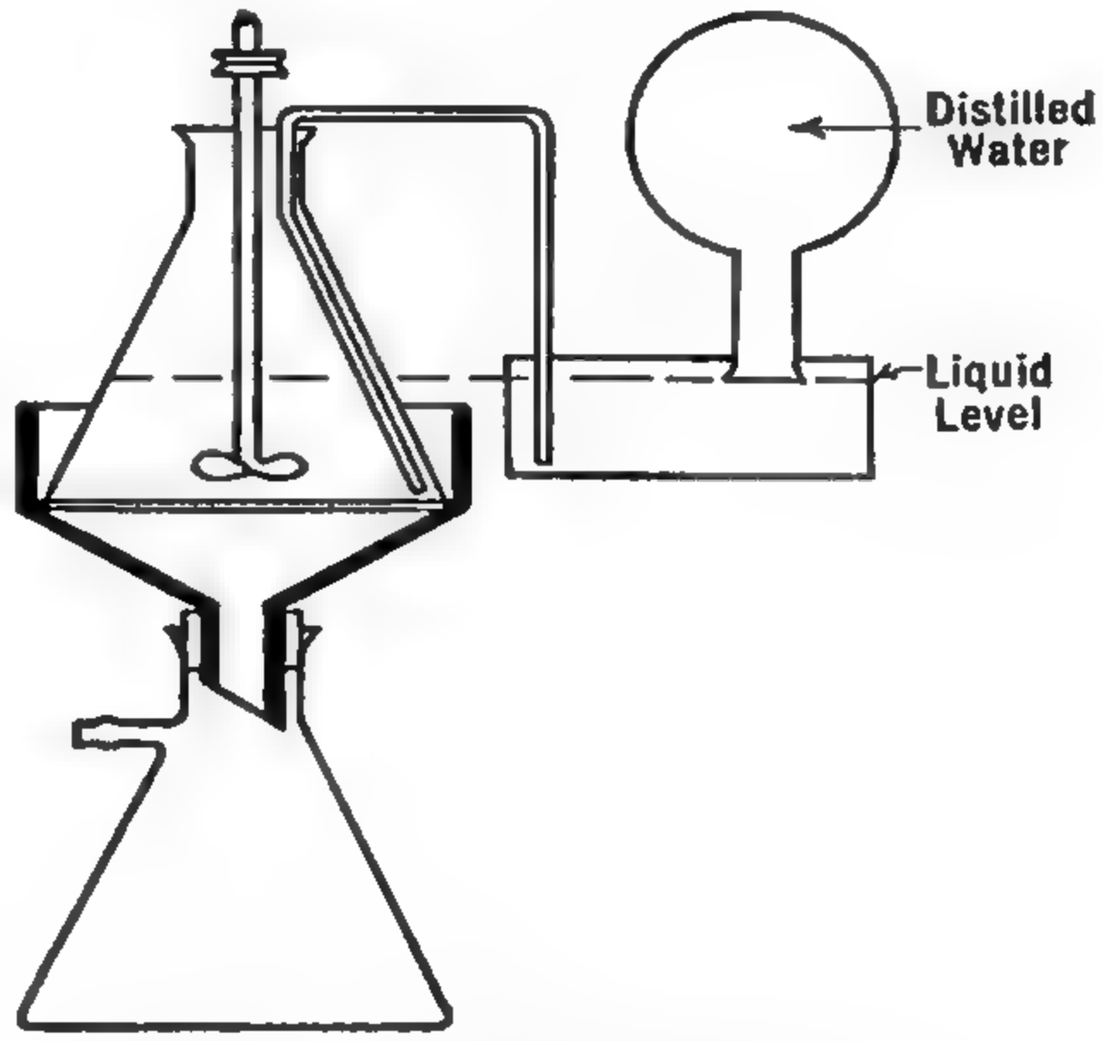
Commercial preparations of invertase, in solid form or in solution, are available on the market. Some of these can be used directly for analytical purposes. Others require purification or concentration or both. This may be accomplished by the ultrafiltration method described above, or by dialysis, followed by evaporation in vacuo at a temperature not exceeding 40° C.

The sucrose determination is made in accordance with the following directions of the Association of Official Agricultural Chemists.[70]

(*a*) *Direct reading.* Dissolve the double-normal weight of the substance (52 g.), or a fraction thereof, in water in a 200-ml. volumetric flask; add the necessary clarifying agent, avoiding any excess; shake, dilute to the mark with

[70] " Methods of Analysis, A. O. A. C.," 5th ed., pp. 493–494, 1940.

water, mix well, and filter, keeping the funnel covered with a watch glass. Reject the first 25 ml. of the filtrate. If a lead clarifying agent was used, remove the excess lead from the solution when sufficient filtrate has collected by adding anhydrous sodium carbonate, a little at a time, avoiding any excess; mix well and filter again, rejecting the first 25 ml. of the filtrate. (Instead of weighing 52 g. into a 200-ml. flask, two 26-g. portions may be diluted to 100 ml. each, and treated exactly as described. Depending on the color of the product, multiples or fractions of the normal weight may be used, and the results reduced by calculation to the basis of 26 g. in 100 ml.) Pipette one 50-ml. portion of the lead-free filtrate into a 100-ml. flask, dilute with water to the mark, mix well, and polarize in a 200-mm. tube. The result, multiplied by 2, is the direct reading (P of formula given below) or polarization before inversion. (If a 400-mm. tube is used, the reading equals P.) If there is a possibility of mutarotation, allow the solution to stand overnight before taking the reading, or, if the result is desired immediately, add a little dry sodium carbonate to make the solution just distinctly alkaline to litmus, and take several readings at intervals until a constant value is obtained.



(*Reproduced with permission from Ind. Eng. Chem., 16, 171.*)

FIG. 204. Reynolds's ultrafiltration apparatus.

(*b*) *Invert reading.* First determine the quantity of acetic acid necessary to render 50 ml. of the lead-free filtrate distinctly acid to methyl red indicator; then to another 50 ml. of the lead-free solution in a 100-ml. volumetric flask, add the requisite quantity of acid and 5 ml. of the invertase preparation, fill the flask with water nearly to 100 ml., and let stand overnight (preferably at a temperature not less than 20°). Cool, and dilute to 100 ml. at 20°. Mix well and polarize at 20° in a 200-mm. tube. If in doubt as to the completion of the hydrolysis, allow a portion of the solution to remain for several hours and again polarize. If there is no change from the previous reading, the inversion is complete. Carefully note the reading and temperature of the solution. If it is necessary to work at a temperature other than 20°, which is permissible within narrow limits, complete the volumes and make both direct and invert readings at the same temperature. Correct the polarization for the optical activity of the invertase solution and multiply by 2. Calculate the percentage of sucrose by the following formula:

$$S = \frac{100\ (P - I)}{142.1 + 0.073\ (m - 13) - t/2}$$

in which

S = percentage of sucrose.
P = direct reading, normal solution.
I = invert reading, normal solution.
t = temperature at which readings are made.
m = g. of total solids in 100 ml. of the invert solution read in the polariscope.

Determine the total solids as percentage by weight and multiply this figure by the density at 20°.

(*c*) *Rapid inversion at* 55–60° C. If more rapid inversion is desired, proceed as follows: Prepare the sample as directed under (*a*), and to 50 ml. of the lead-free filtrate in a 100-ml. volumetric flask add glacial acetic acid in sufficient quantity to render the solution distinctly acid to methyl red. The quantity of acetic acid required should be determined before pipetting the 50-ml. portion, as described under (*b*). Then add 10 ml. of invertase, mix thoroughly, place the flask in a water bath at 55–60° C., and allow to stand at that temperature for 15 minutes with occasional shaking. Cool, add sodium carbonate until distinctly alkaline to litmus paper, dilute to 100 ml. at 20°, mix well, and determine the polarization at 20° in a 200-mm. tube. Allow the solution to remain in the tube for 10 minutes, and again determine the polarization. If there is no change from the previous reading, the mutarotation is complete. Carefully note the reading and the temperature of the solution. Correct the polarization for the optical activity of the invertase solution and multiply by 2. Calculate the percentage of sucrose by the formula given under (*b*).

The concentration coefficient 0.073 in the formula used for calculating the sucrose was found experimentally by Paine and Balch.[71] The same authors give the fundamental Clerget constant at 20° C. as 132.12, but this has been rounded off to 132.1.

Determination of the Activity of Invertase Preparations. The invertase used in the method of the Association of Official Agricultural Chemists must have a certain minimum activity in order to complete the inversion during the time specified. For purely analytical purposes the following simple test is usually adequate:[72]

Dilute 1 ml. of the invertase preparation to 200 ml. Transfer 10 g. of sucrose (granulated sugar) to a sugar flask graduated at 100 ml. and 110 ml., dissolve in about 75 ml. of water, add 2 drops of glacial acetic acid, and dilute to the 100-ml. mark. To the 100 ml. of sugar solution add 10 ml. of the dilute invertase solution and mix thoroughly and rapidly, noting the exact time at which the solutions are mixed. At the termination of exactly 60 minutes make a portion of the solution just distinctly alkaline to litmus paper with

[71] *J. Am. Chem. Soc.*, **49**, 1019 (1927); in a later investigation Jackson and McDonald found a concentration coefficient of 0.0824 (*J. Assoc. Official Agr. Chem.*, **22**, 580).

[72] " Methods of Analysis, A. O. A. C.," 5th ed., p. 492, 1940.

anhydrous sodium carbonate and polarize in a 200-mm. tube at 20°. If the invertase solution is sufficiently active, the alkaline solution will polarize approximately 31° Ventzke without correcting for the dilution to 110 ml. and the optical activity of the invertase solution.

If more exact information on the activity of the invertase preparations is desired its determination must be based on the kinetics of the reaction. Inversion by invertase, unlike that by acid, is not a unimolecular reaction. The value of the unimolecular velocity constant increases slightly with the time, and invertase preparations from different sources give different reaction curves even when the initial sucrose concentration and other conditions are the same. A number of reaction formulas have been proposed, but none of them has been found to apply to all cases. Nelson and Hitchcock[73] give the following formula for what they term "normal" invertase preparations from yeast, the most common source of invertase:

$$t = \frac{1}{n} \log \frac{100}{100 - p} + 0.002642\, p - 0.000008860\, p^2 - 0.0000001034\, p^3$$

where t is the time, p the per cent inversion, and n a constant which is a measure of the amount of active invertase present. Invertase preparations that do not conform to this formula are considered abnormal.

Although the unimolecular reaction law does not hold for invertase inversion, the variations in the velocity coefficient k calculated with its use are usually so small that an average value determined for several time periods is sufficiently exact for practical purposes, and the methods generally employed for the determination of invertase activity are all based on measurements of the k value for a unimolecular reaction.

Method of the Association of Official Agricultural Chemists.[74] This method is as follows:

Dilute 1 ml. of the invertase solution to 200 ml. at 20°; place in a constant-temperature bath at 20°; and when the solution has attained the latter temperature pipette 20 ml. of it into a flask containing 200 ml. of a sucrose solution (10 g. per 100 ml. concentration) that has been previously made distinctly acid to methyl red (corresponding to *p*H approximately 4.6) by the addition of acetic acid and also brought to a temperature of 20° in the same bath. Mix thoroughly and promptly, and note the time at which the invertase solution was added. Keep the sucrose-invertase mixture in the constant-temperature bath; remove portions at the end of 15, 30, and 45 minutes,

[73] *J. Am. Chem. Soc.*, 43, 2632 (1921); for a discussion of invertase action see Nelson, *Chem. Rev.*, 12, 1 (1933).

[74] "Methods of Analysis, A. O. A. C.," 5th ed., p. 492, 1940.

render each portion just distinctly alkaline to litmus paper with anhydrous sodium carbonate immediately after removing; and polarize at 20°. Correct all polarizations for the polarization of the invertase solution.

The value of the velocity constant k is calculated, for each of the three time intervals, by the unimolecular reaction formula

$$k = \frac{1}{t} \log \frac{a}{a - x} = \frac{\log a - \log (a - x)}{t}$$

In a solution containing 10 g. of sucrose in 110 ml., a is the total change in rotation upon complete inversion, that is, the sum of the initial rotation R_0 and the numerical value of the levorotation after inversion, $0.318\,R_0$; hence $a = 1.318\,R_0$. The value of x is the fall in the polarization, that is, the initial rotation R_0 minus the rotation R_t after time t; hence $(a - x)$ equals $1.318\,R_0 - (R_0 - R_t)$, or $0.318\,R_0 + R_t$. Rounding off 0.318 to 0.32, and 1.318 to 1.32, we obtain the formula

$$k = \frac{\log 1.32\,R_0 - \log (R_t + 0.32\,R_0)}{t}$$

R_0 is calculated by multiplying the polarization of the sucrose solution (10 g. in 100 ml.) by 10/11, and correcting for the polarization of the invertase solution; t is the number of minutes from the time the invertase and sucrose solutions were mixed until inversion was stopped by the addition of alkali. Logarithms on the base 10 are used.

Example. The sucrose solution gave a polarization of 38.4°. $R_0 = 38.4 \times 10/11 = 34.9$, the polarization of the invertase solution being negligible. Log 34.9 = 1.54283, log 1.32 = 0.12057; log $1.32\,R_0$ = 1.66340. The value of $0.32\,R_0$ = 11.17. The reading obtained after 15 minutes was 34.05°. $R_t + 0.32\,R_0 = 34.05 + 11.17 = 45.22$; log 45.22 = 1.65533. Log $1.32\,R_0$ − log $(R_t + 0.32\,R_0)$ = 1.66340 − 1.65533 = 0.00807. This, divided by t (15 minutes), gives k = 0.000538. Similarly, the readings after 30 and 45 minutes were 33.10 and 32.35, giving k values of 0.000576 and 0.000549, respectively. The average k is therefore 0.000554.

The average k value must be multiplied by 200 to obtain the K value of the original invertase preparation, which was diluted 1 : 200. The result is $K = 0.1108$. The minimum K value required in the method of the Association of Official Agricultural Chemists for the determination of sucrose is 0.1. If the K value found is much higher than 0.1, the invertase solution is diluted proportionately before use.

Gore[75] has proposed to simplify the calculations and to reduce the manipulations, by diluting the original invertase 50-fold instead of

[75] *Ind. Eng. Chem., Anal. Ed.*, **4**, 367 (1932).

200-fold, and by taking only one reading, after an elapsed time of 50 minutes. In this case the dilution and the number of minutes cancel each other, so that the K value for the original preparation is obtained directly by the expression: $\log 1.32\ R_0 - \log\ (R_t + 0.32\ R_0)$. This method is sufficiently exact for invertase preparations to be used in industrial processes, but the method of the Association of Official Agricultural Chemists should preferably be employed in analytical work.

Time Value, Invertase Unit, and Invertase Value. The chemist who prepares his own invertase needs to know not only the activity of his preparations, but also the quantity of enzyme in a given weight or volume. Since invertase has not yet been obtained in the pure state, and since it is not certain that chemically pure invertase from different sources would have the same sucrose-inverting power, it is necessary to use arbitrary units. Willstätter and Kuhn[76] proposed to define the "invertase unit" as the quantity of invertase giving a "time value" of 1 minute, in 50 mg. of the invertase preparation. The determination of the number of invertase units, or the "invertase value," is based on the fact that the quantity of invertase required to hydrolyze a given quantity of sucrose is, over a considerable range, inversely proportional to the time.

The "time value" is the time, in minutes, required to reduce the rotation of a sucrose solution to zero, under specified conditions. This corresponds to 75.76 per cent inversion if complete inversion is accepted to produce a rotation of —32° for the normal weight at 20° C. The time value for reduction to zero rotation is designated as t_0.

The usual procedure,[77] which is a modification of the method originally proposed by O'Sullivan and Tompson,[78] is to dissolve 0.05 g. of the invertase preparation in 5 ml. of 0.05 N disodium phosphate solution, and to add this solution to 20 ml. of a sucrose solution containing 20 g. in 100 ml., at a temperature of 15.5° C. The mixture is allowed to stand at this temperature, and the number of minutes elapsed when the solution reaches zero rotation is measured. Mutarotation must be completed by making the solution alkaline before the readings are taken. In practice it is easier to determine the velocity constant k for several time intervals, average the k values found, and to calculate t_0 by means of the formula

$$t_0 = \frac{0.61542}{k}$$

[76] *Ber.*, **56B**, 509 (1923).

[77] Oppenheimer, "Die Fermente und ihre Wirkungen," 5th ed., Vol. 5, p. 770, 1928.

[78] *J. Chem. Soc.*, **57**, 834 (1890).

which is derived as follows:

$$k = \frac{\log 1.32\, R_0 - \log (0 + 0.32\, R_0)}{t_0}$$

$$k = \frac{\log 1.32 - \log 0.32}{t_0} = \frac{0.61542}{t_0}$$

The invertase value is calculated as shown in the following example:

Twenty milliliters of an invertase solution with 50 mg. dry substance have been obtained. The k value, determined with 1 ml. of the solution (2.5 mg. dry substance), is found to be 0.0362, corresponding to a t_0 of 17.0 minutes. The time value is $17.0 \times 2.5 \div 50 = 0.85$ minute, and 0.85 ml. of the invertase solution contains 1 invertase unit. The number of invertase units in 20 ml., or the invertase value, is therefore 23.6.

Time Value and Invertase Value for 50 Per Cent Inversion. The time value based on reduction to zero rotation has a logical foundation for the hydrolysis of sucrose, but not for that of other sugars, such as maltose or lactose, where the direction of the rotation is not reversed. Willstätter and Steibelt[79] suggested therefore a unit which may be used for any carbohydrate or enzyme. The time value in this system is the time required to hydrolyze 50 per cent of the substrate under specified conditions, and the enzyme unit is the quantity of enzyme in 1 g. of enzyme preparation, which causes 50 per cent hydrolysis in 1 minute. Weidenhagen[80] has proposed to apply this system to the testing of commercial invertase preparations, and in the case of liquid products to refer the invertase value to 100 ml. of the invertase preparation, instead of to 1 g. dry substance. The determination of the time value t_{50} is carried out as follows:

Dissolve 2.375 g. sucrose in a 50-ml. volumetric flask, and add 5 ml. of acetate buffer of pH 4.6 (strongly buffered invertase solutions may require as much as 10 ml.; with others less than 5 ml. is sometimes sufficient). Add enough water to leave room for the enzyme solution, and place the flask in a water bath at 30° C. Pipette a measured quantity of the invertase solution into the flask, and start the stop watch when half of the enzyme solution has been added. Make up to the mark, mix well, and replace the flask in the water bath. After definite time intervals pipette 5 ml. of the mixture into 10 ml. of twice-normal sodium carbonate solution, mix well, and polarize at 20° C. The initial rotation R_0 under these conditions is +2.90° V., and the rotation after complete inversion —0.93° V. The velocity constant is

[79] *Z. physiol. Chem.*, **111, 157 (1920); 115, 211 (1921).**
[80] *Z. Ver. deut. Zucker-Ind.*, **82, 992 (1932).**

calculated by the usual formula, and the results found for the different time intervals are averaged. The time value for 50 per cent inversion is found by dividing the average k value into log 2 (0.30103), since in this case $x = a/2$.

The number of invertase units in 100 ml. of the invertase preparation is then calculated by the formula $100 \div (t_{50}\, i)$, where i is the milliliters of invertase solution used in the determination of the velocity constant.

Example. Using 0.2 ml. of an invertase preparation, an average k value of 0.0276 was found under the specified experimental conditions. Then the time value t_{50} for 50 per cent inversion is $0.30103 \div 0.0276 = 10.9$ minutes, and the number of invertase units or the invertase value for 100 ml. of the invertase preparation is $100 \div (10.9 \times 0.2) = 45.9$.

Weidenhagen's method gives very low polariscopic readings, and the error in the readings is greatly multiplied in the calculation of the velocity constant. The large amount of alkali used to complete the mutarotation may cause rapid destruction of invert sugar. It would be better to dilute with 10 ml. of water and to add just enough dry sodium carbonate to give a distinctly alkaline reaction to litmus paper, as prescribed in the method of the Association of Official Agricultural Chemists.

The Inverton. It has been found by various investigators that the initial rate of inversion is the most reliable basis for ascertaining the concentration of an invertase preparation. Johnston, Redfern, and Miller[81] have confirmed this and suggested a new invertase unit, termed the inverton and defined as the quantity of invertase which inverts 5 mg. of sucrose at zero time, at a temperature of 25° C., under the following experimental conditions: A solution containing 0.1 to 1 g. of the invertase in 100 ml. total volume is prepared, depending on the strength of the sample. Of this diluted sample, 25 ml. is pipetted into a 200-ml. volumetric flask. The flask is placed in a thermostat at 25° C., and, when the temperature of the solution reaches this point, 25 ml. of a solution containing 100 g. of sucrose and 50 ml. of acetate buffer of pH 4.6 in 1 liter total volume are added from a fast-running pipette during constant agitation, the time being noted. After exactly 30 minutes the reaction is stopped and the mutarotation completed by the addition of 0.5 ml. of 15 N ammonia. The reading is taken at 25° C. after not less than 5 minutes or more than 2 hours. The initial rotation is determined upon a mixture of 25 ml. of the diluted sample, 0.5

[81] *Ind. Eng. Chem., Anal. Ed.*, **7**, 82 (1935).

ml. of 15 *N* ammonia, and 25 ml. of the sucrose solution, mixed in the order named.

Johnston, Redfern, and Miller determined the milligrams of sucrose inverted under these conditions at various time intervals, for several invertase preparations, and it was found from the curves that the number of milligrams of sucrose inverted at zero time is strictly proportional to the concentration of the enzyme. Next, the relation between the number of invertons in 25 ml. of the diluted invertase solution and the number of milligrams of sucrose inverted under the same conditions in a time interval of 30 minutes was determined, and the following equations were found, in which I is the number of invertons and S the milligrams of sucrose:

$$\log I = 1.0667 \log S - 2.3368$$

for less than 1025 mg. of sucrose, and

$$\log I = 0.0004289\, S + 0.4490$$

for more than 1025 mg. of sucrose.

These formulas were found to be valid for several preparations of invertase. If it is desired to ascertain whether a given invertase sample behaves normally it is only necessary to determine the number of invertons at different dilutions and to see whether they are proportional to the concentration.

Calculations. Since the original sucrose solution contains 2500 mg., the number of milligrams inverted in 30 minutes is found from the equation

$$S = \frac{2500\,(R_0 - R_{30})}{1.292\,R_0}$$

where R_0 is the initial rotation, R_{30} the rotation after 30 minutes, and $1.292\,R_0$ is the total change in polarization upon complete inversion when all the readings are taken at 25° C. The coefficient 1.292 is based on Hudson's Clerget divisor $141.7 - 0.5\,t$.

Substituting the value for S from the above formula in the formulas for $\log I$, and simplifying, we obtain

$$\log I = 1.0667 \log \frac{R_0 - R_{30}}{R_0} + 1.1690$$

for values of I up to 7.5, and

$$\log I = 0.8299\, \frac{R_0 - R_{30}}{R_0} - 0.4490$$

for values of I above 7.5.

The calculations may be eliminated by the use of nomograms, given in the paper of Johnston, Redfern, and Miller.

If the rotations are measured at the standard temperature of 20° C. and the Clerget divisor is corrected for concentration according to the formula of Paine and Balch (p. 434), the factor 1.292 changes to 1.315, and the formulas for log I become:

$$\log I = 1.0667 \log \frac{R_0 - R_{30}}{R_0} + 1.1609$$

for values of I below 7.5, and

$$\log I = 0.8154 \frac{R_0 - R_{30}}{R_0} - 0.4490$$

for values of I above 7.5.

As all the methods for determining and expressing the activity of invertase preparations are based on the unimolecular reaction formula and differ mainly in experimental details, such as temperature and concentration, the multiplicity of terms and procedures has created much uncertainty and confusion. It is very desirable that a standard method be established by international agreement.

CRITICAL COMPARISON OF DIFFERENT INVERSION METHODS

Under the auspices of the Association of Official Agricultural Chemists, Zerban and collaborators[82] made a critical study of several typical inversion methods applied to solutions containing known amounts of sucrose in mixture with invert sugar, reversion products of invert sugar, amino compounds (aspartic acid and asparagine), and ash.

Four inversion methods were employed: (1) the acid hydrolysis method of the Association, patterned after Schrefeld's procedure (p. 407); (2) the invertase method of the Association (p. 432); (3) Jackson and Gillis's general method II (p. 420); and (4) Jackson and Gillis's method IV (p. 420). The inversions were carried out mostly at room temperature, but in some of the work higher temperatures were also employed, viz., 67–69.5° C. in (1), 55° in (2), and 60° in (3) and (4).

It was soon recognized that various commercial materials which originally contain invert sugar but have been heated during the manufacturing operations give evidence of the presence of reversion products. This is an additional source of error in all inversion procedures employing acid, because under the conditions of the analysis these

[82] *J. Assoc. Official Agr. Chem.*, 8, 384 (1925); 9, 166 (1926); 10, 183 (1927); 11, 167 (1928); 12, 158 (1929); 13, 188 (1930); 14, 172 (1931).

reversion products are partly hydrolyzed at room temperature as well as at the higher temperatures specified above, and thus indicate more sucrose than is actually present. The invertase method is the only one which is free from this source of error.

The results obtained in these investigations may be summarized as follows:

(1) The solution used for the direct polarization must have the same dry-substance concentration as the solution used for inversion.

(2) The Clerget divisor to be used must be based on the dry-substance concentration, and not on the difference between the direct and the invert polarizations.

(3) It is preferable wherever possible to carry out the inversion at room temperature, because at high temperatures slight variations in the time used may have an appreciable effect on such reactions as the destruction of invert sugar in the presence of strong acid, on the hydrolysis of reversion products, and on the interaction between invert sugar and amino compounds. While glucose alone in pure aqueous solution is quite stable even at 50° C., fructose suffers decomposition at that temperature or above. In the presence of hydrochloric acid, fructose shows incipient decomposition at as low a temperature as 30° C.; again glucose is much more stable, even at 50°. When asparagine or aspartic acid is added to fructose, the fructose is markedly attacked at 30° and above; with glucose, decomposition begins to show at 50°. The simultaneous presence of hydrochloric acid slows up the reaction between the reducing sugars and amino compounds.

(4) The invertase method is the only one of the four methods compared which may be depended upon to give reliable results, provided that inversion is carried out at room temperature. In the presence of amino compounds the sucrose result of the invertase method at room temperature as specified by the Association (p. 433) is slightly in error because the solutions used for the direct reading and the invert reading do not have the same hydrogen-ion concentration. This error, which is very small, may be corrected by adjusting both solutions to the same *p*H before the readings are taken.[83]

(5) The sucrose result by Jackson and Gillis's method II is increased by reversion products hydrolyzed under the conditions of the analysis. Amino compounds, on the other hand, tend to decrease the sucrose

[83] Poe, Cooley, and Witt have shown that large quantities of certain preservatives such as formaldehyde, boric acid, borax, or sodium salicylate, give low invert readings, either by a direct effect on the rotation of invert sugar, or by inactivating the enzyme. But the small quantities of preservatives actually used for food preservation have no ill effect on the determination of sucrose by the invertase method. (*J. Ind. Eng. Chem., Anal. Ed.*, 5, 309 [1933].)

result, by destruction of fructose. Unless the neutralization with ammonia is carried out with the greatest care so that no local overheating takes place, some fructose is destroyed, especially in the presence of amino compounds.

(6) The sucrose result by Jackson and Gillis's method IV is increased by the hydrolysis of reversion products in the same way as in method II. Asparagine or aspartic acid lowers the sucrose result, owing to the large difference in hydrogen-ion concentration between the neutral solution for the direct reading and the strongly acid solution for the invert reading. It was shown by Ambler[84] that practically all cane products contain appreciable quantities of amino compounds, and for this reason Jackson and Gillis's method IV does not give correct results for the sucrose in such materials.

(7) The difference between the sucrose result by Jackson and Gillis's method II and that by the invertase method gives an approximate measure of the reversion products hydrolyzed by hydrochloric acid under the conditions of the analysis.

(8) The difference between the sucrose result by Jackson and Gillis's method II and that by method IV gives an approximate measure of the amino compounds present.

(9) The plain acid method may give any kind of a result, high, low, or correct within the limits of error, depending on the relative proportions between the different constituents of the mixture analyzed.

(10) In mixtures of known amounts of sucrose with a practically sucrose-free, low-purity product containing 13.74 per cent ash on the basis of the dry substance, the ash itself had no noticeable effect on the Clerget divisor, for any of the four methods investigated. This is contrary to the observations of other investigators. Wohl, Staněk, Herles, and Sázavský have reported an increase in the Clerget divisor due to molasses ash, while Schlemmer noted a decrease.

CLARIFICATION OF SOLUTIONS FOR THE DETERMINATION OF SUCROSE BY THE DOUBLE POLARIZATION METHOD

Basic lead salts are the reagents most generally employed for the clarification of solutions for double polarization. Exactly equal quantities must be added to both solutions for the direct and the invert reading so that they may be as nearly alike as possible in composition. The best way to accomplish this is to add the lead salt or solution to the original solution of the product, filter, remove any excess lead, filter again, and then to use one portion of the final filtrate for the direct reading and an equal portion for inversion.

[84] *Intern. Sugar J.*, 29, 437 (1927).

The principal lead salts used for clarification are basic lead acetate, Herles's basic lead nitrate, and sometimes neutral lead acetate. The principal deleading agents are sodium carbonate, potassium or sodium oxalate, sodium phosphate, and sodium sulfate, more especially the first two.

When lead salts and deleading agents are added in the form of solutions, not only is it necessary to make up the solution to a larger volume after deleading, but also there results an appreciable volume error. This error could be corrected for by making up to a slightly larger volume, but then the volume of the precipitate would have to be determined separately in each case, which is usually not practicable. However, the volume error can be largely reduced by the use of Horne's dry subacetate of lead or dry neutral lead acetate, and of dry deleading agents.

Several widely used methods of clarification are given as examples.

Method of the Association of Official Agricultural Chemists.[85] The solutions are prepared as follows, for inversion by either acid or invertase. The double-normal weight of the substance, or a fraction thereof, is dissolved in water in a 200-ml. flask. The necessary quantity of clarifying agent, neutral lead acetate solution, lead subacetate solution, or Herles's basic lead nitrate solution is added, avoiding an excess. Alumina cream may also be used, with or without lead reagent. The solution is well shaken, diluted to the mark, again shaken, and filtered, the funnel being kept covered with a watch glass. The first 25 ml. of the filtrate is rejected. When sufficient filtrate has collected any excess lead is removed by the addition of anhydrous sodium carbonate, a little at a time, again avoiding an excess. The solution is again shaken and filtered, the first 25 ml. of filtrate being rejected. One 50-ml. portion of the final filtrate is diluted to 100 ml. and used for the direct reading; another 50-ml. portion is inverted in a 100-ml. flask, made up to volume and polarized as already described.

Method of Jackson and Gillis, with Dry Lead Subacetate.[86] These authors recommend the following procedure: "Prepare the normal solution of the substance to be analyzed or a solution of such fractional normality as the nature of the material and the sensibility of the saccharimeter will permit. Clarify with the minimum quantity of dry basic lead acetate. Shake thoroughly and filter. If desired, the solution may at this point be freed from lead; but if this is done, the deleading agent must be added to the whole filtrate. Finely powdered potassium oxalate in minimum quantity is added until precipitation

[85] "Methods of Analysis, A. O. A. C.," 5th ed., p. 493, 1940.

[86] *Bur. Standards Sci. Paper* 375, p. 184, 1920.

is complete. Filter. If this procedure is omitted, the lead is precipitated satisfactorily by the chlorides added later." It should be remarked that the last sentence applies only to the acid inversion method and its modifications. Jackson and Gillis state that, even when the clarified filtrate contains an excess of lead, it is nevertheless neutral in reaction, and that the hydrogen-ion concentration is sufficiently low not to change the rotation which amino compounds show at neutral reaction. This rule is not general, however. Beet molasses is often distinctly alkaline before and after clarification with lead salts, and it is always safest to remove the excess lead.

Method of Java Sugar Experiment Station for Clarifying Molasses with Basic Lead Nitrate.[87] To 35.75 g. molasses (13 g. $\times$ 2.5 $\times$ 1.1), dissolved in water and washed into a 250-ml. flask add 30 ml. of a solution of lead nitrate (600 g. plus 1 liter of water) and 30 ml. of sodium hydroxide solution (80 g. dissolved to 1 liter total volume). Add water up to the lower end of the neck of the flask, without agitation. Roll the flask gently between the hands to allow air bubbles to rise. Remove foam with ether, and fill to the mark. Mix well and filter through a dry paper into a dry 100–110-ml. flask, discarding the first runnings. Cover the funnel with a watch glass during filtration. Collect filtrate up to the 100-ml. mark, add 9 ml. of 30 per cent aluminum sulfate solution, and fill with water to the 110-ml. mark. Add a little kieselguhr and filter. The saccharimeter reading of the filtrate gives the direct polarization. Fifty milliliters of the same filtrate is inverted in a 100-ml. flask by Steuerwald's method. The concentration of the solution for inversion is thus only one-half of that for the direct polarization, which is wrong in principle.

The Czechoslovakian Committee for Uniform Methods[88] prescribes clarification with another modification of Herles's basic nitrate (p. 327); 20 ml. of each of the two separate solutions is added for each normal weight of molasses. The filtrate is practically free from lead and requires no deleading.

In this connection it should be remarked that, with substances high in ash and requiring large amounts of basic lead for clarification, the usual quantity of hydrochloric acid prescribed in the acid hydrolysis methods (5 ml. acid of sp. gr. 1.188 or its equivalent) may be insufficient for inversion on account of the formation of chlorides and the liberation of weak organic acids. In such cases it is usual to employ an additional 1 to 2 ml. of acid of the above strength. This further

[87] *Java Sugar Expt. Sta., Bull.*, 11, 27 (1927).
[88] *Z. Zuckerind. čechoslovak. Rep.*, 53, 53 (1928).

addition naturally changes the value of the Clerget constant, and Saillard has pointed out that in all such cases the constant should be determined upon pure sucrose under exactly similar conditions.

Pellet's Deleading Method with Sulfurous Acid.[89] Instead of the reagents above mentioned, concentrated sulfurous acid (prepared by saturating water with sulfur dioxide) has been proposed by Pellet for deleading. This reagent has certain advantages, for, in addition to precipitating excess of lead, it neutralizes any free alkalinity and at the same time acts as a bleach upon any coloring matter which might darken the solution for reading. The sulfur dioxide has even been added to excess for deleading, sufficient quantity (10 ml.) of the solution being taken to complete the volume from 100 to 110 ml. This excess does no harm, as the acid in the cold is a very weak inverting agent and has no immediate depressing influence upon the direct polarization. This excess of sulfurous acid has also the advantage of preventing the troublesome after-darkening which frequently results from the inverting action of hydrochloric acid. Ogilvie[90] claims as another advantage an equalizing effect in the conditions before and after inversion in that both direct and invert polarizations are made in acid solution. It is evident, however, that the total quantity of acid is not the same in both cases and that these different amounts of acid will exercise a variable influence upon the rotation of fructose, amino compounds, etc.

An objection against sulfur dioxide as a deleading agent is the very troublesome character of the lead sulfite precipitate which, on account of its finely divided colloidal condition, is very apt to pass through the filter. Agitating the solution with paper pulp, infusorial earth (kieselguhr), or kaolin previous to filtration has been recommended as a means of securing a clear filtrate.

Other Deleading Agents. Cross[91] proposed the use of dry powdered oxalic acid for deleading; the filtrate is used both for the direct polarization and for inversion with hydrochloric acid. This makes it possible to use a sufficient excess of lead subacetate to secure a very light-colored filtrate, and to remove this excess again without introducing metallic ions. But the same objection applies to this procedure as to all others where the composition of the two solutions for double polarization is different.

Oxalic acid may, however, be used as a deleading agent in connection with the invertase method of the Association of Official Agricultural

[89] *Ann. chim. anal. appl.*, **14**, 248 (1909).
[90] *Intern. Sugar J.*, **13**, 145 (1911).
[91] *Louisiana Expt. Sta. Bull.*, **135**, 25 (1912).

Chemists, instead of the sodium carbonate specified. The acid serves the dual purpose of removing excess lead and of adjusting the *p*H of the solution to the optimum value for inversion with invertase. Another deleading reagent which has the same effect is ammonium dihydrogen phosphate. But when either of them is used the polarization of the solution for the direct reading must not be unduly delayed because of the danger of incipient inversion, and on the other hand the reading must not be taken too soon, in order that the mutarotation of any invert sugar present in the original product may be completed. These difficulties militate against the use of these reagents in practical sugar work.

Effect of Lead Clarification on Sucrose Determination. The most direct way to obtain information on this subject is to analyze a product that is light enough in color to require no clarification and to make parallel analyses after the addition of increasing quantities of lead subacetate, followed by deleading. A low-purity product is best for this purpose because with such a product the non-sucrose constituents exert their greatest effect. Zerban and coworkers[92] analyzed two refiner's sirups, containing about 35 and 43 per cent sucrose, respectively, which answered the above requirement. The invertase method was employed in this work. The solutions were clarified with lead subacetate solution and deleaded with powdered potassium oxalate. Even with twice the quantity of lead required to precipitate the impurities, the same percentage of sucrose, within 0.15 per cent, was found as without clarification. This would indicate that the volume error is compensated for by other errors, principally that due to the effect of the increasing quantity of dissolved salts upon the Clerget constant. While these results are interesting, they cannot be applied indiscriminately to other low-purity products.

Clarification and Sucrose Determination in Sweetened Condensed Milk. For some materials, especially animal products, lead salts do not effect sufficient purification, and recourse must be had to other clarifying agents. As an example, the method of sucrose determination adopted by the Association of Official Agricultural Chemists[93] for the analysis of sweetened condensed milk is cited. This product is clarified with a solution of mercuric nitrate, prepared by adding to 220 g. of yellow mercuric oxide 300 to 400 ml. of water, and then gradually a sufficient quantity of nitric acid (about 140 ml.) to form a clear solution, avoiding an excess. The solution is diluted to 800 to 900 ml., and 10 per cent sodium hydroxide solution is added slowly

[92] *J. Assoc. Official Agr. Chem.*, **14**, 172 (1931).

[93] "Methods of Analysis, A. O. A. C.," 5th ed., p. 290, 1940.

and with constant shaking until a slight permanent precipitate is obtained. The solution is diluted to 1 liter and filtered. Upon prolonged standing the solution tends to form a deposit, and it is necessary to add sodium hydroxide again until a permanent precipitate is formed, and to refilter.

One hundred grams of the sweetened condensed milk is weighed into a 500-ml. flask, diluted to the mark with water, and thoroughly mixed. Fifty milliliters of the emulsion is pipetted into a 100-ml. flask, 25 ml. of water is added, and then 5 ml. of the mercuric nitrate solution. The mixture is well shaken and at once neutralized to litmus by the addition of 0.5 N sodium hydroxide solution with continued shaking. The volume is completed to the mark; the mixture is again thoroughly shaken and filtered. The sucrose is determined in the filtrate by the Schrefeld method, with inversion at room temperature. Both direct and invert readings must be corrected for the volume occupied by the precipitated protein (1 g. = 0.8 ml.) and by the fat (1 g. = 1.075 ml.). The sucrose is calculated by the formula:

$$S = \frac{100\ (P - P')}{132.35 - 0.5\ (t - 20)} \times \frac{26}{W}$$

where W is the weight of sample taken (10 g.).

Decolorization of Clarified Solutions. The use of decolorizing agents, like bromine water or potassium bromide plus chloramine-T, in lieu of clarifying agents, has already been mentioned (see p. 424). It frequently happens that solutions clarified with lead salts are still too dark to be read comfortably in the saccharimeter; particularly the darkening which results from the action of the hydrochloric acid upon coloring matter or other impurities is often so great as to cause difficulties in reading the solution for the invert polarization. In such cases a number of expedients may be used.

(1) *Use of a 100-mm. or 50-mm. Tube.* Since shortening the length of the observation tube always necessitates a corresponding multiplication of any errors of observation, this method is to be used only as a last resort.

(2) *Decolorization by Means of Animal or Vegetable Char.* Animal charcoal or bone black should never be used upon solutions for direct polarization on account of its great adsorptive power for sucrose. The effect of animal char on invert sugar is less pronounced if it has been purified by washing with dilute hydrochloric acid and water, and drying. The various vegetable decolorizing carbons which have been recommended instead of blood or bone char differ widely in their adsorptive properties. All of them do adsorb sugars, although less from

impure than from pure solutions. Our knowledge of what actually happens when any of these carbons is used for decolorization is very limited. They should be resorted to solely in case of absolute necessity, and in minimum quantity. The best way to use them is to filter the solution to be decolorized in several successive small portions through the carbon placed on a filter, and to discard the first two or three portions, using the remainder for the invert polarization.

(3) *Decolorization by Means of Reducing Agents — Zinc Dust, Sodium Sulfite, Etc.* A large number of reducing agents have been used for decolorizing acid solutions of invert sugar. Zinc dust has been employed frequently for this purpose, the destruction of coloring matter being due to the nascent hydrogen generated by the action of the hydrochloric acid upon zinc. The powdered metal is added to the solution to be decolorized in successive small amounts, thus preventing a too violent evolution of gas with loss of solution.

Sodium sulfite and bisulfite have also been employed for decolorizing acid invert sugar solutions. In this case the bleaching agent is the sulfur dioxide liberated by the action of the hydrochloric acid.

The use of zinc and sodium sulfite as decolorizing agents is not attended with serious danger, provided only that the minimum amounts are employed.

(4) *Increasing the Intensity of the Illumination in the Saccharimeter.* This is by far the best method to be used with solutions too dark to be read under ordinary circumstances. It may be accomplished by widening the half-shadow angle of the polarizer, which is possible with some of the modern instruments, or by employing a stronger electric bulb as the light source, or by both these measures together.

GENERAL RELIABILITY OF THE DOUBLE POLARIZATION METHOD

Although any of the methods of double polarization give perfectly reliable results upon pure sucrose, it is evident that they have serious limitations when applied to the examination of impure products. The only procedure that can be depended upon to give exact results within the limit of error of saccharimetric readings is that employing purified, highly active invertase for hydrolysis, provided that the readings are made at 20° C. Zerban[94] has shown this to be true for products not requiring clarification, by the analysis of known mixtures of sucrose, invert sugar, salts, and amino compounds. It is true even for refiner's sirups clarified with lead subacetate solution and deleaded; here the volume error is evidently compensated for by other errors.

[94] *Proc. 9th Session, Intern. Com. Uniform Methods of Sugar Analysis*, p. 35, 1936.

But when acid is used for hydrolysis, or if the readings are made at temperatures widely varying from the normal of 20° C., the chemist need not expect to obtain upon products containing a mixture of sucrose with reducing sugars, salts, and organic impurities an accuracy much better than 0.5 per cent; in certain cases the error may exceed 1 per cent. These methods, therefore, at best give only an approximation, the degree of exactness depending not only upon the care and skill of the chemist, but also upon the nature of the substance being analyzed. The introduction of excessive refinements in the methods has usually proved a thankless labor and is not to be recommended. The employment, for example, of a Clerget factor elaborated to the fifth decimal (as in Tuchschmid's formula, p. 405) is of no possible value in practical work.

In employing any of the numerous Clerget modifications it is always advisable for the chemist to establish his own factor for the particular conditions of the analysis. This is best done by making a blank determination upon pure sucrose, or, better still, upon a mixture of pure sucrose with approximate amounts of the accompanying substances which are known to occur in the product undergoing examination. By so doing the chemist will gain an idea of the reliability of his method such as can be secured in no other way.

APPLICATION OF THE DOUBLE POLARIZATION METHOD TO THE DETERMINATION OF OTHER SUGARS IN THE PRESENCE OF SUCROSE

When sucrose occurs in the presence of another sugar the percentage (Z) of the accompanying sugar may be determined as follows:

If P is twice the direct polarization of the half-normal weight (13.013 g.) of substance, and S the percentage of sucrose by the invertase method of double polarization, then $P - S$ is the polarizing power of the accompanying sugar. The percentage Z may then be determined as on p. 302, by dividing the value $100(P - S)$ by the polarizing power of the accompanying sugar (Table LI). The calculation may also be expressed in general terms by the equation

$$Z = \frac{66.5\ (P - S)}{[\alpha]_D^{20}}$$

in which 66.5 is the specific rotation of sucrose and $[\alpha]_D^{20}$ that of the accompanying sugar. The method of calculation may be illustrated by several examples.

Example I. A 60° Brix sirup containing sucrose and glucose gave a direct polarization of +58.0 and an invert polarization of −8.33 at 20° C. Required the percentages of sucrose and glucose. According to the formula given on p. 433

$$\text{Per cent sucrose} = \frac{100\,[58 - (-8.33)]}{132.1 + 0.073\,(7.8 - 13)} = \frac{6633}{131.72} = 50.36$$

$$\text{Per cent glucose} = \frac{66.5\,(58 - 50.36)}{53.1} = 9.57, \text{ uncorrected for concentration}$$

Example II. A 66.7° Brix sirup containing sucrose and invert sugar gave a direct polarization of +52 and an invert polarization of −21 at 20° C. Required the percentages of sucrose and invert sugar. According to the formula on p. 433,

$$\text{Per cent sucrose} = \frac{100\,[52 - (-21)]}{132.1 + 0.073\,(8.67 - 13)} = \frac{7300}{131.78} = 55.40$$

$$\text{Per cent invert sugar} = \frac{66.5\,(52 - 55.40)}{-20.8} = 10.87, \text{ uncorrected for concentration.}$$

Example III. A sweetened condensed milk containing 70 per cent total solids gave a direct polarization of +51.50 and an invert polarization of −4.20 at 20° C. Required the percentages of sucrose and lactose. According to the formula on p. 433,

$$\text{Per cent sucrose} = \frac{100\,[51.50 - (-4.20)]}{132.1 + 0.073\,(9.1 - 13)} = \frac{5570}{131.82} = 42.25$$

$$\text{Per cent lactose} = \frac{66.5\,(51.50 - 42.25)}{52.5} = 11.72$$

The percentages of sugars calculated in this manner have of course no greater degree of accuracy than the Clerget sucrose determination. With impure products clarified by means of basic lead compounds there may be an appreciable error due to the occlusion of reducing sugars in the lead precipitate.

Estimation of Commercial Glucose in Sirups. A method based on the same principle has been used by Leach[95] for the approximate determination of commercial glucose added to sirups which normally contain principally sucrose, but no or little invert sugar, such as maple sirup, high-grade cane sirup, and preserving sirup. This method has been adopted by the Association of Official Agricultural Chemists[96] and is carried out as follows: The percentage of sucrose S is determined by the usual inversion method. Then, if a is the direct po-

[95] *Bur. Chem. Bull.* 65, p. 48.

[96] "Methods of Analysis, A. O. A. C.," 5th ed., p. 498, 1940.

larization of the normal-weight solution, the percentage of commercial glucose solids, G, in the sirup is calculated by the formula

$$G = \frac{100\ (a - S)}{211}$$

where 211 is the average polarization of the normal weight of commercial glucose solids. The method gives only approximate results, even in the absence of invert sugar, because of the varying polarization of commercial glucose solids. If the sucrose is determined by hydrolysis with hydrochloric acid another error arises from the effect of the acid on the polarization of the commercial glucose solids (see p. 478).

The result for G may be converted into terms of commercial glucose sirup of any Baumé by dividing G by the corresponding percentage of solids and multiplying by 100.

Method of Juckenack and Pasternack.[97] This, still simpler method is applicable to sirups which contain sucrose, invert sugar, and commercial glucose, but no other optically active constituents. If the sucrose is inverted and the total solids in the sirup are determined, the commercial glucose solids can be calculated from the direct polarization of the sirup. If $[\alpha]_D^{20}$ is the specific rotation of the sirup solids, then the percentage of the commercial glucose solids in the sirup solids, G, is found by the formula:

$$G = \frac{100\ ([\alpha]_D^{20} + 21.5)}{134.1 + 21.5}$$

where the specific rotation of invert sugar is taken as —21.5°, and that of the commercial glucose solids as +134.1°.

If a saccharimeter with the Ventzke scale is used, the inversion is carried out with invertase, and the polarization of the normal weight of commercial glucose solids is called +211°, the formula changes to

$$G = \frac{100\ (a + 32.1)}{211 + 32.1}$$

where a is the polarization of the normal weight of sirup solids.

Example. A sirup consisting of sucrose, invert sugar, and commercial glucose was found to contain 70.5 per cent solids. A quantity of sirup corresponding to the half-normal weight of solids, or 18.46 g. sirup, was inverted with invertase in a 100-ml. flask, made to the mark, and the polarization read at 20° C. in a 200-mm. tube. The reading was +28.7° V., or +57.4° V.

[97] *Z. Untersuch. Nahr. u. Genussm.*, **8**, 10 (1904).

for the normal weight of solids. $G = 100\ (57.4 + 32.1) \div 243.1 = 36.8$ per cent. The percentage of commercial glucose solids in the sirup itself is $36.8 \times 0.705 = 25.9$ per cent.

If the Schrefeld method of inversion (p. 407) is used, the value 32.1 in the formula for G changes to 33.0.

Bruhns[98] has called attention to the fact that the method of Juckenack and Pasternack is subject to considerable error not only through the varying specific rotation of commercial glucose, but also because most of the products to which the method might be applied contain reversion products and other optically active substances. This is particularly true of artificial honeys. Behre[99] has proposed to reduce the figure for the specific rotation of invert sugar in the formula of Juckenack and Pasternack from 21.5 to 20.0, to correct for the reversion products in artificial honey, but this is only an average value, and purely conventional.

With the increasing use of dextrose in food products, to replace part of the sucrose, the methods for the determination of commercial glucose have become partly obsolete.

A method similar to that of Juckenack and Pasternack, but to be applied to natural honeys, is described on p. 480.

Method of Dubois for Determining Sucrose and Lactose in Milk Chocolate. Dubois[100] has applied the Clerget method to the determination of sucrose and lactose in milk chocolate. The usual procedure is somewhat modified in that 100 ml. of water is added to the 26 g. of substance, a correction being afterwards applied for the increase in volume through solution of sugars. A preliminary extraction of the chocolate with petroleum ether to remove fat secures a more rapid solution of sugars.

The Association of Official Agricultural Chemists proceeds in the following manner:[101]

Transfer 26 g. of the sample, prepared by chilling, grating or shaving, and mixing, to an 8-ounce nursing bottle, add about 100 ml. of petroleum ether, shake 5 minutes, and centrifuge. Decant the clear solvent carefully, and repeat the treatment with petroleum ether. Place the bottle containing the defatted residue in a warm place until the petroleum ether is expelled. Add 100 ml. of water and shake until most of the chocolate is detached from the sides and bottom of the bottle. Loosen the stopper and carefully immerse the bottle for 15 minutes in a water bath kept at 85–90° C. (to complete the

[98] *Deut. Zuckerind.*, **59**, 593 (1934).
[99] *Z. Untersuch. Nahr. u. Genussm.*, **43**, 36 (1922).
[100] *Cir.* 66, U. S. Bur. Chem., p. 15.
[101] "Methods of Analysis, A. O. A. C.," 5th ed., pp. 201–202, 1940.

mutarotation of the lactose), shaking occasionally to remove all the chocolate from the sides of the bottle. Remove from the water bath, cool, and add basic lead acetate solution (1.25 sp. gr.) to complete precipitation (5 ml. is usually sufficient). Add water to make a total volume of 110 ml. of added liquid. Mix thoroughly, centrifuge, and decant the supernatant liquid through a small filter. Precipitate the excess of lead with powdered dry potassium oxalate and filter. Dilute sufficient filtrate with an equal volume of water, mix and polarize in a 200-mm. tube at 20° C. Obtain the invert reading at 20° C. after inversion with hydrochloric acid by the Schrefeld method. Multiply both readings by 2 to correct for dilution.

In the original method of Dubois the approximate percentages of sucrose (S) and lactose (L) were first calculated by the following formulas:

$$S = \frac{110\,(P - I)}{C - 0.5\,t} \quad \text{and} \quad L = \frac{(1.10\,P) - S}{0.79}$$

where C is the Clerget constant, t the temperature of polarization, and 0.79 the ratio of the specific rotation of lactose to that of sucrose (see p. 302). The approximate grams of total sugars (G) was calculated from S and L, and the volume (X) of solution estimated by the formula $X = 110 + (G \times 0.62)$, in which 0.62 is the increase in volume caused by dissolving 1 g. of sugar in water. The corrected percentages of sucrose and lactose were then found as follows:

$$\text{True per cent sucrose} = \frac{S \times X}{110}\,; \text{ true per cent lactose} = \frac{L \times X}{110}$$

The employment of an expansion factor, as in the above method, is permissible only in case of water-free substances and where no other ingredients than sugars are dissolved. The factor 0.62 is not absolutely correct for all concentrations, as is seen from the following table:

Sucrose Dissolved in 100 ml. Water at 20° C.	Volume of Resulting Solution	Increase in Volume through Solution of 1 g. Sucrose	Sucrose Dissolved in 100 ml. Water at 20° C.	Volume of Resulting Solution	Increase in Volume through Solution of 1 g. Sucrose
grams	ml.	ml.	grams	ml.	ml.
1	100.51	0.506	26	115.98	0.614
2	101.12	0.560	50	130.94	0.619
5	102.96	0.592	100	162.37	0.624
10	106.07	0.607	200	225.82	0.629

The error attending the use of the factor 0.62 upon dilute solutions is small, but to avoid it altogether Seeker, Shanley, and Lourie[102] suggested calculating the increase in volume directly from the polarizations. If X is the correction due to the increase in volume by dissolved sucrose and lactose, then

$$S = \frac{(P - I)\,(110 + X)}{143 - 0.5\,t} \quad \text{and} \quad L = \frac{P\,(1.1 + 0.01\,X) - S}{0.79}$$

The value of X is calculated from the following equation[103]

$$X = 0.62 \times 26 \left[\frac{(P - I)\,(1.1 + 0.01\,X)}{143 - 0.5\,t} + \frac{0.01\,P\,(1.1 + 0.01\,X) - \dfrac{(P - I)\,(1.1 + 0.01\,X)}{143 - 0.5\,t}}{0.79} \right]$$

To simplify the calculations, the expression $\dfrac{(P - I)}{143 - 0.5\,t}$ is termed d. Then the equation for X changes to

$$X = 16.12 \left[d\,(1.1 + 0.01X + \frac{P\,(1.1 + 0.01X) - 100\,d\,(1.1 + 0.01X)}{79} \right]$$

Solving for X,

$$X = \frac{0.2244\,(P - 21\,d)}{1 - 0.00204\,(P - 21\,d)}$$

which value is substituted in the equations given above for calculating S and L.

This modification of the Dubois method was used for many years by the Association of Official Agricultural Chemists. But Fitelson[104] found in 1931 that, although sucrose can be estimated very accurately by this method, more exact results for lactose are obtained by copper reduction, and the method has been changed accordingly.

A method similar to that of Dubois has been described by Rinck and Kaempf,[105] and tables to simplify the calculations have been given by Rinck and Müller.[106]

[102] *J. Assoc. Official Agr. Chem.*, 3, 491 (1920).
[103] Private communication from J. W. Sale.
[104] *J. Assoc. Official Agr. Chem.*, 15, 551 (1932); 16, 564 (1933); 17, 377 (1934).
[105] *Z. Untersuch. Lebensm.*, 59, 81 (1930).
[106] *Z. Untersuch. Lebensm.*, 65, 626 (1933).

APPLICATION OF THE CLERGET PRINCIPLE TO THE DETERMINATION OF RAFFINOSE

The principle of the Clerget inversion method may be applied to the analysis of any optically active substance whose specific rotation undergoes a known change with a special method of treatment. The most common application of the principle, outside of sucrose, is in the determination of the trisaccharide raffinose, which occurs in beet-sugar products and various plant substances.

The hydrolysis of raffinose with hydrochloric acid, under the conditions prescribed for the Clerget inversion, proceeds very closely according to the equation:

$$\underset{\substack{\text{Raffinose}\\ [\alpha]_D^{20} = +104.5 \text{ (for raffinose hydrate before hydrolysis)}}}{C_{18}H_{32}O_{16}} + H_2O \rightarrow \underbrace{\underset{\substack{d\text{-Fructose}\\ [\alpha]_D^{20} = -92}}{C_6H_{12}O_6} + \underset{\substack{\text{Melibiose}\\ [\alpha]_D^{20} = +143}}{C_{12}H_{22}O_{11}}}_{[\alpha]_D^{20} = +53.5 \text{ (for raffinose hydrate after hydrolysis)}}$$

The specific rotation of raffinose decreases during the hydrolysis from +104.5 for the hydrate to +53.5, which corresponds to that of a molecular mixture of fructose and melibiose. The normal weight of raffinose for the Ventzke scale, corresponding to 26.026 g. of sucrose ($[\alpha]_D^{20} = 66.5$) in 100 ml., is 16.562 g. for the hydrate ($[\alpha]_D^{20} = 104.5$) and 14.051 g. for the anhydride ($[\alpha]_D^{20} = 123.17$). These amounts of raffinose, polarizing 100° V., show after hydrolysis, following exactly the procedure of Schrefeld, a polarization of +51.40° V. at 20° C., or a decrease of 48.60° V., which calculated to the weight of raffinose reading 1° V. (0.16562 g. hydrate or 0.14051 g. anhydride) is 0.486° V. The calculation of pure raffinose by the acid hydrolysis method may then be expressed as follows:

$$R = \frac{P - P'}{0.486}$$

in which R is the percentage of raffinose, P the polarization of the normal weight of product before hydrolysis, and P' the polarization at 20° C. of this normal weight after hydrolysis.

The same formula applies if the polarization is measured on the Bates-Jackson scale and a normal weight of 16.545 g. raffinose hydrate or 14.037 g. anhydride is used.

APPLICATION OF THE INVERSION METHOD TO MIXTURES OF SUCROSE AND RAFFINOSE

Raffinose is almost always associated in nature with sucrose, and since sucrose undergoes inversion simultaneously with the hydrolysis

of raffinose, the formula previously given for the calculation of raffinose has but little practical value. Creydt,[107] however, showed that it was possible to combine the equations for the calculation of raffinose and sucrose, and in this way obtain formulas which can serve for the estimation of the two sugars in mixtures. The original formulas of Creydt were based upon the old Clerget process of inversion and have now been largely replaced by formulas worked out for the Herzfeld[108] modification (p. 406). The method of establishing these formulas may be understood from the following:

If the sucrose normal weight (26.026 g.) of a substance containing S per cent of sucrose and R per cent of raffinose (anhydride) is dissolved to 100 ml. and polarized in a 200-mm. tube, the polarization of the sucrose in degrees Ventzke will be represented by S and the polarization of the raffinose by $1.852\,R$ (the value 1.852 being the ratio 26.026/14.051 of the normal weight for raffinose anhydride to that for sucrose). The direct polarization P (the sum of the sucrose and raffinose polarizations) is represented then by the formula

$$P = S + 1.852\,R, \text{ whence } R = \frac{P - S}{1.852} \text{ and } S = P - 1.852\,R \qquad (1)$$

If the sucrose normal weight of the above substance is inverted according to Schrefeld's method and polarized at 20° C., the invert polarization of the sucrose will be represented by $-0.33\,S$ (since 1° V. sucrose before inversion reads −0.33° V. at 20° C. after inversion). In the same manner the polarization of the raffinose after hydrolysis will be $1.852\,R \times 0.514$ (since, for a raffinose solution reading 100° V., each 1° V. before hydrolysis reads +0.514° V. at 20° C. after hydrolysis by Schrefeld's method). The invert polarization P' (the sum of the sucrose and raffinose invert polarizations) is represented then by the formula

$$P' = -0.33\,S + 1.852\,R \times 0.514 \qquad (2)$$

By substituting the quantity $\frac{P - S}{1.852}$ of equation 1 for R in equation 2, we obtain the formula

$$P' = -0.33\,S + 0.514\,(P - S)$$

whence

$$S = \frac{0.514\,P - P'}{0.844} \qquad (3)$$

[107] *Z. Ver. deut. Zucker-Ind.*, 37, 153 (1887).
[108] *Z. Ver. deut. Zucker-Ind.*, 40, 194 (1890).

S having been calculated from P and P', the value of R is obtained from equation 1,

$$R = \frac{P - S}{1.852}$$

By substituting the quantity $P - 1.852\,R$ of equation 1 for S in equation 2, we obtain the formula

$$P' = -0.33\,(P - 1.852\,R) + 0.9519\,R$$

whence

$$R = \frac{0.33\,P + P'}{1.563} \qquad (4)$$

By formula 4 the raffinose may be calculated at once from the direct and invert polarizations. As formulas 3 and 4 do not correct for the variations in P' with the changes in concentration of sucrose and raffinose the results are not absolutely exact. The following example illustrates the use of the formulas.

A beet molasses, free of reducing sugar, gave a direct polarization of +49.75° V. and an invert polarization of −14.80° V.

$$\text{By formula 3, per cent sucrose} = \frac{0.514 \times 49.75 - (-14.80)}{0.844} = 47.83$$

$$\text{By formula 1, per cent raffinose} = \frac{49.75 - 47.83}{1.852} = 1.04$$

$$\text{By formula 4, per cent raffinose} = \frac{0.33 \times 49.75 + (-14.80)}{1.563} = 1.04$$

Correction of Raffinose Formula for Changes in Temperature. The determinations of sucrose and raffinose by the preceding formulas must be carried out at exactly 20° C. If the analysis is made at other temperatures, the formulas must be modified. Browne and Gamble[109] found the direct polarization of raffinose, when the solution was made up and polarized at other temperatures than 20° C., to diminish 0.00034 per 1° V. for each 1° C. increase in temperature, which is but slightly higher than the temperature variation for sucrose. The temperature correction for the direct polarization of both sucrose and raffinose may, therefore, be taken as $1 - 0.0003\,(T - 20)$. Equation 1, when the solution for the direct polarization is made up and polarized at temperature T, would then become

$$P = S\,(1.006 - 0.0003\,T) + 1.852\,R\,(1.006 - 0.0003\,T) \qquad (5)$$

[109] *J. Ind. Eng. Chem.*, **13, 793 (1921).**

Several formulas have been worked out for correcting the invert polarizations of sucrose and raffinose for changes in temperature. Herles[110] found a solution of raffinose which read 100° V. upon the saccharimeter before inversion to read, after inversion by Herzfeld's method, +51.24 when the inverted solution was made up and polarized at 20° C. and to read +47.24 when the inverted solution was made up and polarized at 0° C., which corresponds to a difference of 0.20 polarization for 1° C. Browne and Gamble[111] found a solution of raffinose which read 100° V. at 20° C. before inversion to read, after inversion by Schrefeld's process, +53.54 when the inverted solution was made up and polarized at 32° C. and to read +49.61 when the inverted solution was made up and polarized at 10° C., which corresponds to a difference of 0.18° V. for 1° C. change in temperature. The temperature correction for the invert polarization of a solution of raffinose reading 100° V. at 20° C. before inversion is therefore $+47.8 + 0.18\,t$, which, calculated to a solution of raffinose reading 1° V., would be $+0.478 + 0.0018\,t$. The temperature correction, under similar conditions per 1° V. direct reading, for the invert polarization of sucrose, employing Schrefeld's process of inversion, is $-0.43 + 0.005\,t$. Equation 2, when the solution for the invert polarization is made up and polarized at temperature t, would then become

$$P' = S\,(-0.43 + 0.005\,t) + 1.852\,R\,(+0.478 + 0.0018\,t) \qquad (6)$$

Substituting in equation 6 the value of R from equation 5, we obtain

$$S = \frac{P\,(0.478 + 0.0018\,t) - P'\,(1.006 - 0.0003\,T)}{(0.908 - 0.0032\,t)\,(1.006 - 0.0003\,T)} \qquad (7)$$

Substituting in equation 6 the value of S from equation 5, we obtain

$$R = \frac{P\,(0.43 - 0.005\,t) + P'\,(1.006 - 0.0003\,T)}{(1.681 - 0.0059\,t)\,(1.006 - 0.0003\,T)} \qquad (8)$$

When T and t are each 20° C., equations 7 and 8 become necessarily the same as equations 3 and 4.

Jackson and Gillis's Raffinose Formulas. These authors have calculated raffinose formulas[112] based on the Clerget constants found for their methods I and II (p. 420). Herzfeld's value of +51.24° V. for the polarization of the normal weight of hydrolyzed raffinose is accepted as correct, in both these methods, but Herzfeld's figure of −32.66° for the polarization of the invert sugar is replaced by

[110] *Z. Zuckerind. Böhmen,* 13, 559 (1888/89).
[111] *J. Ind. Eng. Chem.,* 13, 795 (1921).
[112] *Bur. Standards Sci. Paper* 375, p. 177, 1920.

—33.25° in method I, and by —33.91° in method II. In the latter case it is assumed that the rotation of hydrolyzed raffinose is the same in the presence of ammonium chloride as in that of hydrochloric acid. When method I is employed, the sucrose is calculated from the equation

$$S = \frac{0.5124\,P - P'}{0.8449}$$

and for method II the equation is

$$S = \frac{0.5124\,P - P'}{0.8515}$$

In either case the raffinose is found as usual from the equation

$$R = \frac{P - S}{1.852}$$

These formulas apply only if the readings are taken at 20° C. and at a concentration of 13 g. sugar in 100 ml. solution before inversion.

To correct for differences in concentration, Jackson and Gillis adopted Herzfeld's coefficient 0.0676 g, where g represents grams dry substance in 100 ml.; to correct for the effect of various temperatures, they employ the coefficients determined by Herles (p. 459) which were in use prior to the redeterminations by Browne and Gamble.

If correction is made for temperature in both the direct and the invert readings, and also for concentration, the formula for method I is changed to

$$S = \frac{[1 + 0.0003\,(t - 20)]\,[P' - P\,(0.4724 + 0.002\,t)]}{0.8961 + 0.000676\,g - 0.003\,t}$$

and that for method II becomes

$$S = \frac{[1 + 0.0003\,(t - 20)]\,[P' - P\,(0.4724 + 0.002\,t)]}{0.9027 + 0.000676\,g - 0.003\,t}$$

The raffinose is again calculated as shown above.

Saillard's Raffinose Formula. Saillard[113] redetermined the rotation of hydrolyzed raffinose under the conditions of the older Clerget procedure where 10 ml. of hydrochloric acid of 22° Baumé (35.21 per cent) is added to 100 ml. solution, and the inversion is effected by gradually heating from 20 to 70° C. in 11 minutes, after which the solution is rapidly cooled to 20° C. and read at this temperature. The rotation of raffinose under these conditions was found to be +51.56° for +100°

[113] *Compt. rend.*, **178**, 2189 (1924).

direct polarization. From this value the following formula is derived:

$$S = \frac{0.5156\,P - P'}{0.8556}$$

$$R = \frac{P - S}{1.852}$$

If the raffinose is to be expressed as the hydrate, the divisor in the last formula is changed from 1.852 to 1.572.

Determination of Sucrose and Raffinose by Hydrolysis with Enzymes. The acid hydrolysis methods described above are subject to the same errors as similar methods applied to the determination of sucrose in the presence of invert sugar and non-sugars. These errors are particularly pronounced in the analysis of final beet molasses which not only may contain considerable amounts of raffinose, but which also are usually high in nitrogenous compounds whose rotation varies greatly with hydrogen-ion concentration, or which may be hydrolyzed by acids to other compounds showing a different rotation. All these errors may be avoided by using enzymes as hydrolyzing agents. The enzymes mainly proposed for this purpose are invertase which splits raffinose into melibiose and fructose, melibiase which forms galactose and glucose from melibiose, and emulsin which produces sucrose and galactose from raffinose. Emulsin has the disadvantage that it also breaks up glucosides which often occur in plant juices, and for this reason only invertase and melibiase are used in practical sugar analysis. Hudson and Harding[114] developed a method in which the raffinose is first hydrolyzed with invertase from top yeast extract, and the melibiose formed is further hydrolyzed with melibiase from bottom yeast extract. The raffinose is then calculated from the difference between the two polarizations. The method required several days for completion because of the weakness of the melibiase solutions used. This difficulty has been completely overcome by Reynolds's ultrafiltration method of preparing enzyme solutions (see p. 431). A practical method for determining both sucrose and raffinose in mixtures by the use of these enzyme solutions was developed by Paine and Balch.[115] It is based on the same principle as the method of Hudson and Harding, but the two hydrolyses are carried on simultaneously. The top yeast extract, containing invertase only, converts the sucrose into glucose plus fructose, and the raffinose into melibiose and fructose; the bottom yeast extract, containing invertase and

[114] *J. Am. Chem. Soc.*, 37, 2193 (1915).

[115] *Ind. Eng. Chem.*, 17, 240 (1925); *J. Am. Chem. Soc.*, 49, 1019 (1927).

melibiase, again splits sucrose into glucose and fructose, but raffinose into glucose, fructose, and galactose. Both sucrose and raffinose may then be derived from two simultaneous equations.

The extracts from top and bottom yeasts are prepared from baker's and brewer's yeasts, respectively, by the method described on p. 431. Both these enzyme preparations are available commercially.

The following directions have been adopted by the Association of Official Agricultural Chemists for carrying out the analysis:[116]

The invertase activity of both the top and bottom yeast extracts should be tested, and that of the top yeast extract should be at least as great as when the invertase is used for the determination of sucrose in the absence of raffinose (see p. 435).

The top yeast extract must be free from melibiase. This may be ascertained by allowing it to act on melibiose, when no change of rotation should be found. To test the melibiase activity of the bottom yeast extract:

Add 2 ml. of the solution to be tested to 20 ml. of a weakly acid melibiose solution polarizing +20.0° V. and allow to stand 30 minutes at about 20°. Then add sufficient sodium carbonate to render the solution slightly alkaline to litmus paper. A preparation suitable for the overnight hydrolysis of solutions containing not more than 0.2 g. of raffinose in 100 ml. should have hydrolyzed 35 per cent of the melibiose present under the conditions mentioned; a preparation suitable for the overnight hydrolysis of solutions containing not more than 0.65 g. of raffinose in 100 ml. should have produced 50 per cent hydrolysis of melibiose; and a preparation suitable for the overnight hydrolysis of solutions containing 0.65–1.3 g. of raffinose in 100 ml. should have hydrolyzed at least 70 per cent of the melibiose present under the above condition. The polarizations that correspond to 35, 50, and 70 per cent hydrolysis of a melibiose solution polarizing, before hydrolysis, +20° V. are: +16.4°, +14.9°, and +12.9° V., respectively.

In the analysis of sugar beet products, weigh the quantity of material specified in the following table, transfer to a 300-ml. volumetric flask, add the quantity of basic lead acetate solution indicated in the table, and dilute to volume at 20°. Mix thoroughly and filter through fluted paper in a closely covered funnel, rejecting the first 25 ml. of filtrate. When sufficient filtrate has collected, remove the lead from the solution by adding ammonium dihydrogen phosphate in as small excess as possible (see table). This condition is readily determined after a little practice by the appearance of the lead phosphate precipitate, which usually flocculates and settles rapidly in the presence of a slight excess of the salt. Mix well and filter, again rejecting at least the first 25 ml. of the filtrate. Make a direct polarization in a 200-mm. tube at 20° unless the solution contains an appreciable quantity of

[116] "Methods of Analysis, A. O. A. C.," 5th ed., pp. 495–497, 1940.

invert sugar, in which case pipette a 50-ml. portion of the lead-free filtrate into a 100-ml. flask, dilute with water to the mark, mix well, and polarize at 20°, preferably in a 400-mm. tube. This reading, calculated to the normal weight of 26 g. in 100 ml. and 200-mm. tube length, is the direct reading (*P*) of the formula given below for polarization before inversion.

QUANTITIES OF SAMPLE AND REAGENTS REQUIRED FOR CLARIFICATION AND DELEADING OF BEET SUGAR-HOUSE PRODUCTS

Material	Quantity per 100 ml.	Basic lead Acetate (55° Brix)	Ammonium Dihydrogen Phosphate
	gram	ml.	gram
Cossettes[a]	13	3	0.2
Pulp	100 ml.[b]	2–4	0.2
Lime cake or sewer[c]	26.5	1.5	....[d]
Thin juice	52	2	0.2–0.3
Thick juice	26	4	0.3–0.4
White massecuite	13 or 26	3 or 6	0.3–0.7
High wash sirup	13 or 26	3 or 6	0.3–0.7
High green sirup	13 or 26	5 or 10	0.3–0.7
Raw or remelt massecuite	13	6	0.3–0.4
Raw or remelt sugar	26	3–4	0.3–0.4
Sugar melter	26	2–3	0.3–0.4
Low wash sirup	13	8–10	0.4–0.5
Low green sirup or molasses	13	10	0.4–0.5
Saccharate cakes and milk (carbonated)	26	4–6	0.3–0.4
Steffen waste and wash waters[c]	78 or 50 ml.	2–3	0.2

[a] Usual method of extraction, 26 g. in 201.2 ml., or better 201.0 ml. (see p. 362).
[b] Dilute to 110 ml.
[c] Neutralize with acetic acid before adding basic lead acetate.
[d] Lime in solution will be partly precipitated by the phosphate, and it is necessary to add sufficient phosphate to complete the precipitation of both the lead and lime salts; hence no definite quality can be specified.

Transfer two 50-ml. portions of the lead-free filtrate to 100-ml. flasks. To one add 5 ml. of invertase solution (top yeast extract) and to the other 5 ml. of invertase-melibiase solution (bottom yeast extract), let stand overnight at atmospheric temperature (preferably not below 20°), dilute to volume, mix well, and polarize at 20°, preferably in a 400-mm. jacketed tube. If a rapid hydrolysis is desired, add 10 ml. of each of the enzyme solutions to the 50-ml. portions of deleaded filtrate in 100-ml. flasks and place in a water bath at 50–55° for 40 minutes. Then add sodium carbonate until the solution is slightly alkaline to litmus paper, dilute to volume at 20°, mix well, and polarize at 20°, preferably in a 400-mm. tube. Correct the invert readings for the optical activity of the enzyme solution and calculate the polarization to that of a normal weight solution of 26 g. in 100 ml.; also calculate the reading to a 200-mm. tube length, if necessary.

The formula for calculating the raffinose from the readings obtained after hydrolysis with top and bottom yeast respectively is derived by Hudson and Harding as follows:

Anhydrous melibiose has a specific rotation of +143. When it is hydrolyzed, 1 g. of it yields 0.527 g. each of glucose, $[\alpha]_D = +52.5$, and of galactose, $[\alpha]_D = +81.0$. The specific rotation changes therefore to +70.4°, a diminution of 72.6°. For 1 g. melibiose in 100 ml. and a 2-dm. tube the change amounts to $\frac{72.6 \times 1 \times 2}{100}$, that is, 1.452 circular degrees, or 4.18° V. Each degree Ventzke therefore indicates 1 ÷ 4.18, or 0.239 g. melibiose, which corresponds to 0.352 g. anhydrous raffinose. Hence the percentage of raffinose is

$$R = \frac{0.352 \times 100\,(A - B)}{26} = 1.354\,(A - B) \qquad (1)$$

where A and B are the polarizations after hydrolysis with top and bottom yeast extract, respectively.

To calculate the sucrose, Paine and Balch proceed in the following manner. As shown on p. 457,

$$S = P - 1.852\,R \qquad (2)$$

If the sucrose polarizes +1° before hydrolysis, the rotation changes to —0.3212° after hydrolysis with invertase; the polarization of the raffinose changes at the same time from +1° to +0.521°.[117] Therefore

$$A = -0.3212\,S + (0.521 \times 1.852)\,R = -0.3212\,S + 0.965\,R \qquad (3)$$

Combining equations 2 and 3, we obtain

$$132.12\,S = 100\,(P - A - 0.887\,R)$$

$$S = \frac{100\,(P - A - 0.887\,R)}{132.12} \qquad (4)$$

Substituting the value of R from equation 1, the formula becomes

$$S = \frac{100\,(P - 2.202\,A + 1.202\,B)}{132.12} \qquad (5)$$

Formulas 1 and 5 are valid for a concentration of 13 g. dry substance in 100 ml. and for a temperature of 20°. If a different concentration is used, the divisor in formula 5 changes to $132.12 + 0.073\,(g - 13)$, where g is grams of dry substance in 100 ml. Paine and Balch did not redetermine the temperature coefficients for the enzyme method. It is best to make all polarizations at 20° C., but if this

[117] Paine and Balch, *J. Am. Chem. Soc.*, **49**, 1019 (1927).

is not done, the temperature corrections established by Browne and Gamble (see p. 459) may be used.

Double-Acid Method of Osborn and Zisch. Although the two-enzyme method of Paine and Balch is admittedly the most accurate and generally applicable procedure for determining sucrose and raffinose in mixtures, the cost of the enzymes is very high, and the procedure requires considerable skill. Zisch[118] has devised a cheaper and simpler method which is based on the observation that the optically active non-sugars in neutral solutions of American beet-sugar products that are free from invert sugar show zero rotation in a strongly acid solution, such as is used in the Clerget method. Osborn and Zisch[119] have developed this method further. Three polarizations are made, one before inversion (P), another after inversion without neutralization of the acid (I), and a third after inversion and neutralization with ammonia (I'). The effect of the neutralization on the rotation of the invert sugar formed was determined for varying concentrations of original sucrose and varying amounts of lead used for clarification, and the values of the necessary correction (K) are shown in Table LXVII. The inversions are carried out by a modification of the method of Walker (p. 412).

TABLE LXVII

Neutralization Corrections for Determining Raffinose by Method of Osborn and Zisch

Concentration[a] Per Cent Sucrose	Ml. of Lead Subacetate (55° Brix) per 100 ml. Solution										
	0	2	4	6	8	10	12	14	16	18	20
	Correction K										
5	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04
10	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.08	0.08
15	0.07	0.08	0.08	0.09	9.09	0.10	0.10	0.11	0.11	0.12	0.12
20	0.09	0.10	0.11	0.11	0.12	0.13	0.13	0.14	0.15	0.16	0.16
25	0.12	0.13	0.13	0.14	0.15	0.16	0.17	0.18	0.19	0.19	0.20
30	0.14	0.15	0.16	0.17	0.18	0.19	0.20	0.21	0.22	0.23	0.24
35	0.16	0.18	0.19	0.20	0.21	0.22	0.24	0.25	0.26	0.27	0.28
40	0.19	0.20	0.22	0.23	0.24	0.26	0.27	0.28	0.30	0.31	0.32
45	0.21	0.23	0.24	0.26	0.27	0.29	0.30	0.32	0.33	0.35	0.36
50	0.24	0.25	0.27	0.29	0.30	0.32	0.34	0.35	0.37	0.39	0.41
55	0.26	0.28	0.30	0.31	0.33	0.35	0.37	0.39	0.41	0.43	0.45
60	0.28	0.30	0.32	0.34	0.36	0.38	0.40	0.42	0.45	0.47	0.49
65	0.31	0.33	0.35	0.37	0.39	0.42	0.44	0.46	0.48	0.50	0.53
70	0.33	0.35	0.38	0.40	0.42	0.45	0.47	0.50	0.52	0.54	0.57
75	0.35	0.38	0.40	0.43	0.45	0.48	0.51	0.53	0.56	0.58	0.61
80	0.38	0.40	0.43	0.46	0.48	0.51	0.54	0.57	0.59	0.62	0.65
85	0.40	0.43	0.46	0.49	0.52	0.54	0.57	0.60	0.63	0.66	0.69
90	0.42	0.45	0.48	0.51	0.55	0.58	0.61	0.64	0.67	0.70	0.73
95	0.45	0.48	0.51	0.54	0.58	0.61	0.64	0.67	0.70	0.74	0.77
100	0.47	0.50	0.54	0.57	0.61	0.64	0.67	0.71	0.74	0.78	0.81

[a] Direct polarization P may be used instead of per cent sucrose.

[118] *Facts About Sugar*, 27, 211 (1932).

[119] *Ind. Eng. Chem., Anal. Ed.*, 6, 193 (1934).

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	Correction K										
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10	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.08	0.08
15	0.07	0.08	0.08	0.09	9.09	0.10	0.10	0.11	0.11	0.12	0.12
20	0.09	0.10	0.11	0.11	0.12	0.13	0.13	0.14	0.15	0.16	0.16
25	0.12	0.13	0.13	0.14	0.15	0.16	0.17	0.18	0.19	0.19	0.20
30	0.14	0.15	0.16	0.17	0.18	0.19	0.20	0.21	0.22	0.23	0.24
35	0.16	0.18	0.19	0.20	0.21	0.22	0.24	0.25	0.26	0.27	0.28
40	0.19	0.20	0.22	0.23	0.24	0.26	0.27	0.28	0.30	0.31	0.32
45	0.21	0.23	0.24	0.26	0.27	0.29	0.30	0.32	0.33	0.35	0.36
50	0.24	0.25	0.27	0.29	0.30	0.32	0.34	0.35	0.37	0.39	0.41
55	0.26	0.28	0.30	0.31	0.33	0.35	0.37	0.39	0.41	0.43	0.45
60	0.28	0.30	0.32	0.34	0.36	0.38	0.40	0.42	0.45	0.47	0.49
65	0.31	0.33	0.35	0.37	0.39	0.42	0.44	0.46	0.48	0.50	0.53
70	0.33	0.35	0.38	0.40	0.42	0.45	0.47	0.50	0.52	0.54	0.57
75	0.35	0.38	0.40	0.43	0.45	0.48	0.51	0.53	0.56	0.58	0.61
80	0.38	0.40	0.43	0.46	0.48	0.51	0.54	0.57	0.59	0.62	0.65
85	0.40	0.43	0.46	0.49	0.52	0.54	0.57	0.60	0.63	0.66	0.69
90	0.42	0.45	0.48	0.51	0.55	0.58	0.61	0.64	0.67	0.70	0.73
95	0.45	0.48	0.51	0.54	0.58	0.61	0.64	0.67	0.70	0.74	0.77
100	0.47	0.50	0.54	0.57	0.61	0.64	0.67	0.71	0.74	0.78	0.81

[a] Direct polarization P may be used instead of per cent sucrose.

[118] *Facts About Sugar*, 27, 211 (1932).

[119] *Ind. Eng. Chem., Anal. Ed.*, 6, 193 (1934).

The lead subacetate solution is prepared according to procedure IV on p. 311, and adjusted to 55° Brix and a *p*H of 7.4 to 7.5 by the addition of litharge or lead acetate as required.

The details of the method are as follows. Five normal weights of sample are transferred to a 500-ml. flask (Kohlrausch type), the necessary quantity of lead subacetate solution (100 ml. for molasses, 70 ml. for raw massecuite, 20 ml. for white massecuite or saccharate sirup) is added, and then water to a total volume of 400 to 450 ml. All air bubbles are removed by placing the flask under a vacuum, a few drops of amyl alcohol being added to break the foam. This de-aeration must be done very carefully, to avoid foam or liquid being carried over into the vacuum line. The volume is completed at 20° C., the solution well shaken and filtered. The filtrate is deleaded with a minimum quantity of dry ammonium dihydrogen phosphate, an excess being carefully avoided. The solution is filtered again, and the filtrate polarized in a 200-ml. tube at 20° C. (*P*). Fifty milliliters each of the filtrate are pipetted into two 100-ml flasks, acidified with 2 drops of hydrochloric acid, density 1.1029 at 20°/4° C., and if the solution is very dark, not more than 0.1 g. of sodium hydrosulfite is added for decolorization. The solution is diluted with 15 ml. of water, and heated to 68 to 69° C. in a water bath kept at 70° C. The flasks are removed from the bath, 10 ml. of the hydrochloric acid is added at once, the flasks are allowed to stand for 2 hours at room temperature, and then adjusted to 20° C. One of the flasks is filled to the mark at 20° C., a little Filter-Cel is added, the solution well mixed, filtered, and polarized in a 400-mm. tube at 20° C. (*I*). To the other flask 1 or 2 drops of 0.2 per cent methyl red indicator solution are added, and the solution is neutralized by adding slowly, with constant whirling of the flask, 6.34 *N* ammonia from a burette. Exactly 1 ml. of ammonia is added in excess. The volume is completed at 20° C., the solution mixed with a little Filter-Cel, filtered, and polarized in a 400-mm. tube at 20° C. (*I'*).

All the filtrations are carried out under battery jars, the inside of which is dampened with water; the jars are placed on wetted rubber mats. This prevents all evaporation during filtration. The first 10 to 20 ml. of each filtrate is discarded.

To calculate the results, the direct polarization *P* is first corrected for the polarizing effect of the non-sugars:

Corrected polarization $P' = P - N$, where $N = I' - I + K$. The value of *K* is taken from Table LXVII. Then (p. 457)

$$\text{per cent sucrose } (S) = \frac{0.514\,P' - I}{0.514 + 0.321 + 0.00009\,P} = \frac{0.514\,P' - I}{0.835 + 0.00009\,P}$$

and

$$\text{per cent raffinose } (R) = 0.54\ (P' - S)$$

In the sucrose formula the expression $0.00009\,P$ represents the concentration correction, based on S. Since there is little difference between S and P in beet products, this substitution is permissible. But it is preferable to use the concentration of dry substance in place of either of them (see p. 442).

The method of calculation is illustrated by the following example.

Found: $P = 50.00$; $I = -14.00$; $I' = -16.00$.

$K = 0.41$ (from table, having used 20 ml. lead acetate and found P to be 50)

$$N = -16.00 - (-14.00) + 0.41 = -1.59$$

$$P' = 50.00 - (-1.59) = 51.59$$

$$S = \frac{(0.514 \times 51.59) - (-14.00)}{0.835 + (0.00009 \times 50)} = 48.26$$

$$R = 0.54\ (51.59 - 48.26) = 1.80$$

If it is necessary to make the polarizations at a temperature other than 20° C., the following formula should be used for calculating sucrose

$$S = \frac{P'\ (0.478 + 0.0018\ t) - I\ (1.006 - 0.0003\ T)}{(0.899 + 0.00009\,P - 0.0032\ t)\ (1.006 - 0.0003\ T)}$$

where T is the temperature of the direct polarization, and t that of the invert polarization.

Finally a correction must be applied to the sucrose and raffinose results for the volume of the lead precipitate. In the case of molasses the results are multiplied by 0.99; the factor for raw massecuite is 0.993, and for white massecuite and saccharate sirup 0.998.

Osborn and Zisch found that this method gives excellent checks with the two-enzyme method of Paine and Balch for American beet factory products free from invert sugar, beyond the carbonatation station, but not for cossettes and diffusion juice. It remains to be seen whether similar products in other countries also show zero rotation of the optically active non-sugars in strongly acid solution, and the double-enzyme method should always be used as a standard of comparison with other methods.

Clarification and Decolorization in Raffinose Determinations. What has been stated on pp. 443–449 applies equally to the clarification of solutions prior to the determination of raffinose. If lead salts

are employed, it is best to add them to the entire solution, then to delead, and to use portions of the final filtrate for the two or three polarizations.

Bone-Black Error in Raffinose Determinations. A source of error peculiar to certain applications of the inversion method for determining raffinose is the increase in levorotation after decolorizing inverted solutions by means of bone black. This error was first studied by Reinhardt,[120] who attributed the phenomenon to the absorption of the highly dextrorotatory melibiose. Reinhardt's explanation is no doubt correct as bone black shows a similar absorptive power for other disaccharides, such as sucrose. Davoll,[121] who has made a detailed study of methods for estimating raffinose, gives the following results upon a mixture containing 94.98 per cent pure cane sugar and 5.02 per cent raffinose hydrate (4.26 per cent raffinose anhydride). The direct polarization for a normal weight of this mixture was +102.48. The invert polarizations for different methods of treatment were as follows:

Method of Treatment	Invert Polarization	Calculated Sugars	
		Raffinose	Sucrose
		per cent	per cent
Without char	−27.00	4.16	94.77
Blood charcoal (purified with acid)	−27.14	4.11	94.87
Animal charcoal (highest purity)	−27.40	3.95	95.16
Animal charcoal (reagent)	−28.00	3.56	95.89

In the above experiments the solutions were shaken 5 minutes with 3 g. of char before filtering. Pouring the solutions in successive portions through the char with rejection of the first runnings (as described on p. 329) would no doubt reduce the error due to absorption considerably.

As a remedy for the error due to the use of bone black Davoll proposes the employment of zinc dust as a decolorizing agent. At the end of the Clerget inversion 1 g. of powdered zinc was allowed to act upon the acid solution at 69° C. for 3 to 4 minutes. Under these conditions the zinc was not found to affect the polarization of the inverted solution.

Osborn and Zisch[122] have found that 0.1 g. of sodium hydrosulfite

[120] *Z. Ver. deut. Zucker-Ind.*, **52, 114 (1902).**
[121] *Proc. Fifth Intern. Congr. Appl. Chem.*, **III, 135.**
[122] *Ind. Eng. Chem., Anal. Ed.*, **6, 193 (1934).**

added to the solutions for the invert readings does not cause a perceptible error, and that it may be used safely.

Raffinose Determination by Previous Precipitation as Saccharate. Schecker[123] also investigated the preparation for analysis of substances containing raffinose and reached the conclusion that the use of either lead subacetate solution or bone black leads to serious errors. The lead volume error alone was found to be 0.75 ml. for the half-normal weight of beet molasses in 100 ml. If purified bone black is used, at least 6 g. is required to bring about the necessary decolorization, and this causes a much greater error than that observed by Reinhardt and by Davoll with 3 g. of char.

To eliminate the effect of the non-sugars on the determination of raffinose in the presence of sucrose, Schecker successfully used precipitation of the two sugars as saccharates, and analysis of the precipitate obtained, which is practically free from non-sugars. A quantity of molasses containing 100 g. sugar by single polarization, 193 g. crystallized barium hydroxide, and 390 g. of water are weighed out in separate portions. The molasses is dissolved with part of the water, and the barium hydroxide in the remainder of it. Both solutions are heated to 90° C. and then mixed together in a metal beaker. The mixture is heated 15 minutes longer at 95–100° C. with constant stirring. The precipitate is filtered off by suction, suspended in water, saturated with carbon dioxide, and refiltered. The solution, which is now free from non-sugars and is of a very light color, is concentrated and analyzed. The first treatment with barium hydroxide precipitates only about one-half of the raffinose. It must be repeated by adding to the mother liquor 100 g. of raffinose-free sucrose, and 90 g. barium hydroxide, adjusting the alkalinity to 18 per cent, and completing the operation as before. About four or five such treatments are required to remove all the raffinose. The method is very time-consuming, but it is free from the errors caused by non-sugars, clarification, etc., and may be resorted to occasionally as a check on other methods. Saillard[124] has used a similar procedure for this purpose.

General Reliability of the Optical Method for Estimating Raffinose. The remarks (p. 449) made upon the limitations of the Clerget method apply with even greater force to the optical determination of raffinose. The simple acid method does not give accurate results, when optically active substances other than sucrose and raffinose are present. Where sucrose occurs with caramelization products, gums, and organic acids, application of the formula may indicate the presence of raffinose

[123] *Z. Ver. deut. Zucker-Ind.*, 72, 1 (1922); 74, 85 (1924).

[124] *Louisiana Planter*, 73, 188 (1924).

when in reality none is present. The two-enzyme procedure of Paine and Balch is much more reliable, but even this method should be used only in the investigation of substances in which raffinose is liable to occur (as sugar-beet products, cotton seed, etc.) and should never be employed, as is sometimes done, as a test for the presence of raffinose in unknown mixtures.

As in the Clerget determination of sucrose the chemist need not expect in the analysis of commercial products for raffinose an accuracy much exceeding 0.5 per cent. The indication of a smaller amount of raffinose than 0.5 per cent is, in fact, not regarded by the best authorities as sufficient to justify reporting its presence (as in raw beet sugars).

Before applying the method to the analysis of unknown products the chemist should first satisfy himself of the presence of raffinose by isolating it in crystalline form, determining its optical rotation and other physical properties, and subjecting it to hydrolysis by acid, invertase, and melibiase. He should also confirm the results of his analysis so far as possible by making blank determinations upon known mixtures. A practical test of this kind is the best means for testing the reliability of the method in particular cases.

APPLICATION OF THE CLERGET PRINCIPLE TO THE DETERMINATION OF MELEZITOSE

Hudson and Sherwood[125] discovered that the rare trisaccharide melezitose occurs occasionally in honeys, being derived from certain mannas upon which bees may feed when floral nectar is scarce. The melezitose may be determined approximately by a combination of acid and invertase hydrolysis. The enzyme does not attack the melezitose, but acid hydrolysis splits it into turanose and glucose, and the rotation of 26 g. in 100 ml. solution falls from +134° V. to +95.5° V. A decrease of 1° V. therefore indicates 0.675 g. melezitose. The method of calculation is best shown by an example taken from the article of Hudson and Sherwood. A sample of honey gave, on the basis of the normal weight, at 20° C., a direct polarization of +27.9°, after inversion with invertase +27.05°, and after the usual acid hydrolysis +17.6°. The decrease in rotation due to the inversion of sucrose alone is therefore 0.85°, and the percentage of sucrose is $85 \div 132.1$, $= 0.64$. The decrease in rotation due to the hydrolysis of sucrose and melezitose is 10.30°, leaving a decrease in rotation, due to melezi-

[125] *J. Am. Chem. Soc.*, **42**, 116 (1920); see also von Fellenberg, *Mitt. Lebensm. Hyg.*, **28**, 139 (1937).

tose alone, of 9.45°. This indicates $9.45 \times 0.675, = 6.4$ g.; $\frac{6.4 \times 100}{26} =$ 24.6 per cent. This method of calculation does not consider the effect of the acid on the rotation of the fructose originally present in the honey and formed from the sucrose, but a correction for it may be applied, if desired.

CHAPTER XI

SPECIAL METHODS OF SACCHARIMETRY

The methods of inversion, described in the previous chapter, are only special instances of a more general course of procedure. It is possible to calculate the percentage of any sugar, provided that its rotatory power, in distinction from that of associated sugars, can be given a definite alteration by some special method of treatment. The changes produced in the rotation of sucrose and raffinose by the action of invertase or acids are but single illustrations of such special methods of treatment. As other examples may be mentioned the determination of sugars by noting the change produced in polarization (1) under different conditions of temperature; (2) after fermenting with yeast; (3) after destroying the optical activity of reducing sugars. Numerous other examples might be given but the three cases cited are sufficient to illustrate the general application of the principle to special problems of saccharimetry.

DETERMINATION OF SUGARS BY POLARIZATION AT HIGH TEMPERATURE

Determination of Invert Sugar by High-Temperature Polarization

The principle of this method is based upon the fact that solutions of pure invert sugar, when heated to a temperature between 85° and 90° C., become optically inactive. This inactivity is due to the lowering in specific rotation of fructose with increase in temperature (p. 272); the specific rotation of glucose being unaffected by temperature, the point of optical inactivity will be the degree at which the polarizing powers of glucose and fructose exactly neutralize each other.

Temperature of Optical Inactivity of Invert Sugar. The temperature of optical inactivity of invert sugar has been variously estimated. Dubrunfaut,[1] who made the earliest measurements of this constant, set the figure at 90° C. Casamajor[2] and Wiley[3] have given 88° C.,

[1] *Compt. rend.*, **42**, 901 (1856).
[2] *Chem. News*, **44**, 219 (1881).
[3] *J. Am. Chem. Soc.*, **18**, 81 (1896).

Lippmann,[4] 87.8° C., Wolf,[5] 87.6° C. and Tuchschmid,[6] 87.2° C. These variations may be due in part to slight experimental errors (such as incipient destruction of sugar at the high temperature) and in part to the influence of concentration. Inasmuch as the $[\alpha]_D$ of glucose varies from +52.5 for a 1 per cent solution to +54.0 for a 40 per cent solution it is evident that the temperatures at which these different polarizations are neutralized must vary somewhat.

The effect of concentration upon the temperature of optical inactivity for invert sugar may be determined by means of the formulas of Gubbe.[7]

I Concentration $[\alpha]_D^{20} = -19.657 - 0.0361\,c$

II Temperature

(20° to 100° C.) $[\alpha]_D^t = [\alpha]_D^{20} + 0.3246\,(t - 20) - 0.00021\,(t - 20)^2$

In Table LXVIII, column *B* gives the $[\alpha]_D^{20}$ of invert sugar, as calculated by formula I, for different concentrations; column *C* gives the grams of invert sugar in 100 ml. necessary to produce a reading of −1° V., as calculated by the expression $\frac{1732}{100[\alpha]_D^{20}}$ (p. 298); column *D* gives the temperature of optical inactivity, as determined by formula II of Gubbe; column *E* gives the variation in degrees Ventzke, produced by 1 g. of invert sugar in 100 ml. for 1° C. difference in temperature and is calculated by the expression $\frac{1}{C\,(D - 20)}$.

TABLE LXVIII

A	*B*	*C*	*D*	*E*
Concentration, Grams Invert Sugar in 100 ml.	$[\alpha]_D^{20}$	Invert Sugar in 100 ml. Corresponding to −1° V. at 20° C.	Temperature of Optical Inactivity	Variation for 1 g. Invert Sugar for 1° C.
grams		grams	° C.	° V.
2	−19.72	0.8783	83.2	0.01801
10	−20.02	0.8651	84.2	0.01800
20	−20.38	0.8499	85.4	0.01799
30	−20.74	0.8351	86.6	0.01798
40	−21.10	0.8209	87.8	0.01797
50	−21.46	0.8071	89.0	0.01796
60	−21.82	0.7938	90.2	0.01795

[4] *Ber.*, **13**, 1823 (1880).
[5] *Oesterr. ungar. Z. Zuckerind. Landw.*, **15**, 331 (1886).
[6] *J. prakt. Chem.* [2], **2**, 235 (1870).
[7] *Ber.*, **18**, 2207 (1885).

For general purposes 87° C. is usually taken as the temperature of optical inactivity for invert sugar. The equations derived by Zerban from Tollens' and Vosburgh's data (see p. 272) cannot be used in this instance because they apply only to a limited range of concentration and temperature.

The application of the method to the determination of invert sugar is easily understood. Since a change of 1° C. produces a constant variation of 0.018° V. for 1 g. of invert sugar in 100 ml., regardless of the concentration, then the grams of invert sugar in 100 ml. of a given solution is found by the formula

$$\text{Invert sugar} = \frac{P' - P}{0.018\ (t' - t)}$$

in which P' = Ventzke-scale reading at higher temperature t', and

P = Ventzke-scale reading at lower temperature t.

The method of applying the formula may best be understood by taking a typical example.

Example. Fifty grams of a solution, containing a mixture of glucose and fructose in unequal amounts, was made up to 100 ml. at 20° C. The polarization was +10.20° V. at 20° C. in a 200-mm. tube.

Fifty grams of the same solution was made up to 100 ml. at 87° C. The polarization was +20.75° V. at 87° C. in a 200-mm. tube. Required the percentage of sugars in the original solution.

$$\text{Invert sugar} = \frac{20.75 - 10.2}{0.018\ (87 - 20)} = 8.75 \text{ g.}$$

$$\frac{8.75}{50} \times 100 = 17.50 \text{ per cent invert sugar}$$

The dextrorotation at 87° C. shows an excess of glucose over the amount necessary to be paired with the fructose for invert sugar. This excess of glucose may be estimated as follows:

Since 1° V. = 0.3226 g. glucose (p. 302) then the grams of glucose corresponding to the dextrorotation at the inactivity of invert sugar is 20.75 × 0.3226 = 6.69 g. (uncorrected for concentration), or 13.38 per cent. To correct for the influence of concentration, the true glucose value of the Ventzke-scale reading +20.75, according to the formula $G = s + 0.02\,s - 0.0002\,s^2$ (p. 300), is 21.08; 21.08 × 0.3226 = 6.80 g. glucose or 13.60 per cent in the original solution. (Strictly, total sugar concentration should be used.)

The percentage of glucose determined by this method of calculation, of course, can be considered as only approximate, for, as shown in Table LXVIII, the temperature of optical inactivity, according to concentration, may be above or below 87° C.

Determination of Commercial Glucose by High-Temperature Polarization

Method of Chandler and Ricketts. The method of high-temperature polarization as first developed in 1880 by Chandler and Ricketts[8] was not employed for determining invert sugar but for detecting the presence and estimating the amount of commercial glucose in cane sugar, molasses, honey, and other products whose sugars, after inversion, consist almost wholly of invert sugar. The material under examination was first inverted to convert any sucrose to invert sugar and then polarized at the temperature of optical inactivity for invert sugar. Any dextrorotation observed at this temperature was attributed to commercial glucose and its percentage estimated by means of an empirical factor.

The factor for converting the readings of the Ventzke sugar scale into grams of commercial glucose depends entirely upon the nature of the product. Commercial glucose, as manufactured in the United States, varies in density from 41° Bé. to 45° Bé. (sp. gr. 1.388 to 1.442) and in specific rotation from about $[\alpha]_D$ +100 to +125 for the liquid product. The grams of commercial glucose corresponding to 1° V. for products of different specific rotation are given in Table LXIX.

TABLE LXIX

$[\alpha]_D$ (for Liquid Product)	Polarization (°V. of 26 g. to 100 ml.)	Grams of Liquid Product in 100 ml. Corresponding to a Polarization of 1° V.	$[\alpha]_D$ (for Liquid Product)	Polarization (°V. of 26 g. to 100 ml.)	Grams of Liquid Product in 100 ml. Corresponding to a Polarization of 1° V.
+125	+188.0	0.1383	+108	+162.5	0.1600
+120	+180.5	0.1440	+105	+157.9	0.1647
+115	+172.9	0.1503	+100	+150.4	0.1729
+110	+165.4	0.1572			

For purposes of analysis the products of $[\alpha]_D$ +114 may be taken as the grade of commercial glucose most commonly used.[9] The chemist should always state the polarizing power of the commercial glucose in terms of which his results are expressed.

The form of polariscope devised by Chandler and Ricketts for high-temperature polarization is shown in Fig. 205. The instrument con-

[8] *J. Am. Chem. Soc.*, 2, 428 (1880).
[9] *J. Assoc. Official Agr. Chem.*, 8, 715 (1925).

sists of an ordinary saccharimeter, with trough removed and replaced by a water bath which is heated from below by means of gas or spirit lamps. The ends of the water bath, before the diaphragms of the analyzer and polarizer, are provided with metallic caps containing small windows of plate glass. The polarization tube, which in its earliest form was constructed of platinum, is completely immersed in

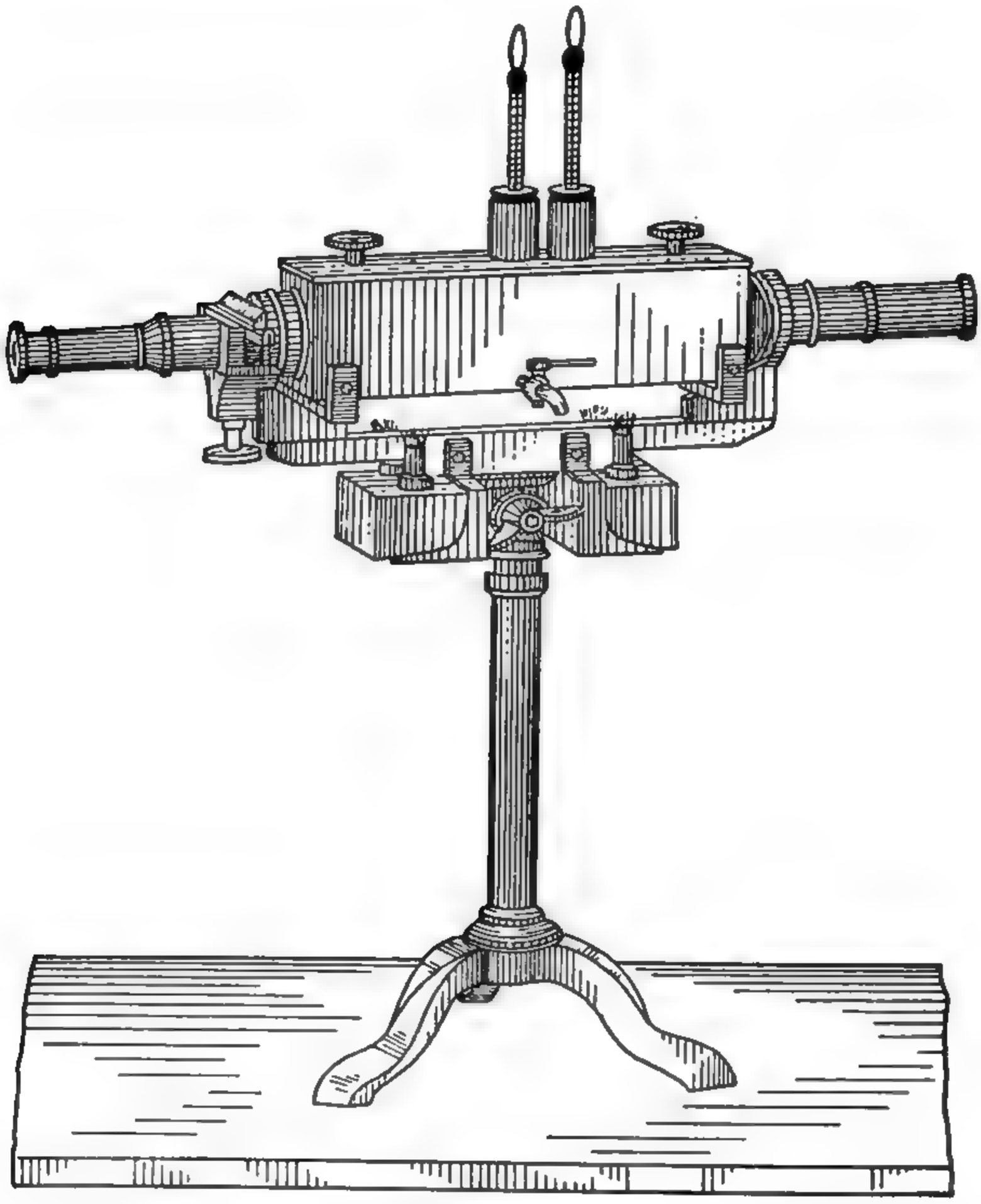


FIG. 205. Chandler and Ricketts's polariscope for high-temperature polarization.

the water of the bath, and rests upon supports opposite the windows and in perfect alignment with the axis of the instrument. The tube is provided with an upright tubule for inserting a thermometer and for receiving any excess of liquid displaced by expansion. The cover of the bath, which fits over the tubule, contains an opening for a thermometer to determine the temperature of the bath.

Similar equipment for taking readings at high temperatures is furnished by various polariscope manufacturers (see p. 166). But the

polarizations may also be made upon an ordinary type of saccharimeter, employing a metal-jacketed tube; the tube may be insulated to advantage by a mantle of asbestos or other non-conducting material. The equipment used at the National Bureau of Standards for high-temperature polarization is shown in Fig. 206. The circulating water is heated in an electrically controlled thermostat, and conveyed through insulated pipes and rubber tubing to the jackets of the polariscope tubes and back again to the thermostat.

(Courtesy of National Bureau of Standards.)

FIG. 206. Equipment for high-temperature polarization.

Method of Leach.[10] Leach's method, as later modified by the Association of Official Agricultural Chemists, for determining commercial glucose in molasses, sirups, honey, etc., is as follows:[11]

Prepare an inverted half-normal solution of the substance by Schrefeld's acid hydrolysis method (see p. 407), except to cool the solution after inversion, make neutral to phenolphthalein with sodium hydroxide solution, slightly acidify with dilute hydrochloric acid (1 + 5), and treat with 5–10 ml. of alumina cream before making up to the mark. Filter, and polarize at 87° C. in a 200-mm. jacketed metal tube, preferably silver. Multiply the reading by 200 and divide by the factor 196 to obtain the quantity of commercial glucose solids polarizing +211° V. (This result may be recalculated in terms of commercial glucose of any Baumé reading desired.)

[10] "Food Inspection and Analysis," p. 644, 1911.
[11] "Methods of Analysis, A. O. A. C.," 5th ed., p. 498, 1940.

In the above method the solution is made up at room temperature and polarized at 87° C. When this is done a correction must be made for the expansion of the solution and consequent lowering of the reading. The best method of making this correction is by means of an empirical test. Thus Lathrop,[12] following the above course of procedure, obtained the following results upon nine samples of commercial glucose.

Sample	Density, ° Baumé	Polarization (26 g. in 100 ml.)			Ratio $\frac{B}{A}$	Ratio $\frac{C}{A}$
		A	*B*	*C*		
		Direct	Invert at 20° C.	Invert at 87° C.		
1	44.17	178.2	176.8	168.0	0.992	0.943
2	43.42	172.8	169.6	160.8	0.982	0.931
3	42.98	169.6	167.6	157.0	0.988	0.926
4	43.02	166.2	163.6	153.0	0.984	0.921
5	43.03	167.2	165.6	155.4	0.990	0.929
6	43.63	175.0	173.4	163.2	0.991	0.933
7	43.00	175.0	173.4	160.4	0.991	0.917
8	42.89	168.6	167.6	156.8	0.994	0.930
9	43.58	170.0	167.4	158.4	0.985	0.932
Average	43.30	171.4	169.4	159.2	0.989	0.929

It is seen that the polarization of commercial glucose is slightly lowered by the action of the acid during inversion, as well as by the expansion of the solution upon heating to 87° C. To correct for both these influences, the polarization value of the glucose is multiplied by 0.929. The Association of Official Agricultural Chemists expresses the result in terms of glucose solids polarizing 211° V. at 20° C., for a weight of 26 g. product in 100 ml. The factor by which the result at 87° C. must be divided is therefore $211 \times 0.929 = 196$. If the result is expressed in terms of glucose sirup polarizing 175° V. at 20° C., the factor is $175 \times 0.929 = 163$.

Example. The half-normal weight of a table sirup, inverted according to Schrefeld's procedure and made up to 100 ml. at 20° C., polarized +32.6° V. at 87° C. Required the percentage of commercial glucose of 42° Baumé and with a polarization of 211° V. for glucose solids.

The percentage of glucose solids equals $\frac{32.6 \times 2 \times 100}{196} = 33.27$ per cent.

[12] *J. Assoc. Official Agr. Chem.*, **8, 715 (1924/25)**.

To convert this result into glucose of 42° Baumé (modulus 145) it must be divided by the percentage of solids corresponding to this figure.

$$\frac{33.27 \times 100}{79.03} = 42.1 \text{ per cent commercial glucose of } 42° \text{ Baumé.}$$

Dextrorotation of Inverted Honey at 87° C. The method of estimating commercial glucose in honeys, sirups, molasses, etc., by polarizing at 87° C., can be regarded as only an approximate one. The chief limitation of the method is the fact that pure honeys, molasses, sirups, etc., are more or less dextrorotatory, after inversion, at 87° C., owing to the presence of gums, dextrins, or similar compounds.

Table LXX, which is taken from the work of Browne,[13] gives the polarization of various samples of American honey at 20° and 87° C., before and after inversion, for 26 g. in 100 ml.

TABLE LXX

Kind of Honey	Number Samples Averaged	Direct Polarization		Invert Polarization		
		20° C.	87° C.	20° C.	87° C.	Difference
Levorotatory Class:		° V.	° V.	° V.	° V.	
Mangrove	1	−24.80	+ 0.50	−27.94	− 0.66	27.28
Mesquit	3	−20.93	+ 4.45	−25.01	+ 2.83	27.84
Sweet clover	4	−17.61	+ 6.80	−22.85	+ 4.70	27.55
Alfalfa	8	−15.10	+ 9.63	−22.99	+ 5.00	27.99
Buckwheat	2	−16.80	+ 8.20	−20.41	+ 5.94	26.35
Cotton	2	−17.50	+ 6.80	−21.01	+ 6.05	27.06
White clover	15	−13.01	+11.65	−17.77	+ 9.25	27.02
Goldenrod	3	−12.33	+10.87	−16.43	+ 9.35	25.78
Dandelion	2	−12.40	+13.00	−18.92	+ 9.51	28.43
Sumac	3	−10.47	+12.53	−14.01	+11.51	25.52
Apple	2	− 8.55	+17.00	−13.73	+12.76	26.49
Basswood	6	− 8.90	+15.05	−12.25	+13.62	25.87
Whitewood	1	− 4.90	+17.80	− 9.68	+15.40	25.08
Dextrorotatory Class:						
Poplar	1	+ 3.60	.....	− 2.53	+20.90	23.43
Hickory	1	+ 7.80	+28.50	+ 3.41	+26.62	23.21
White oak	1	+11.00	+32.30	+ 5.17	+28.60	23.43
Sugar-cane honey dew	1	+17.75	.....	+13.53	+34.76	21.23
Levorotatory honeys	92	−14.73	+10.15	−19.16	+ 7.91	27.07
Dextrorotatory honeys	7	+ 9.43	+32.20	+ 5.47	+27.56	22.09
Average of 50 varieties	99	−13.02	+10.81	−17.41	+ 9.30	26.71

[13] "Chemical Analysis and Composition of American Honeys," *Bull.* 110; U. S. Bur. Chem.

Application of the formula 100 $P/196$ to the invert polarizations at 87° C. would indicate nearly 8 per cent commercial glucose solids in some of the levorotatory and nearly 15 per cent in several of the dextrorotatory honeys.

Browne's Method for Estimating Commercial Glucose in Honey. Browne[14] has modified the application of the high-temperature polarization, for estimating commercial glucose in honeys by taking the difference between the invert polarization at 20° and 87° C. as a basis of calculation. It is seen from Table LXX that, while the invert readings at either 20° or 87° C. are subject to the widest variations, the difference between the polarizations at these two temperatures is a fairly constant quantity for nearly all honeys. The average value of this constant for the 99 samples of honey examined by Browne was 26.7. Since this difference in polarization is due entirely to the percentage of invert sugar in the honey, the addition of any commercial glucose will cause a depression in the polarization difference, which will be proportional to the amount of commercial glucose used but irrespective of its specific rotation. In order to correct for the variations in moisture and non-sugars of pure honey it is better to express the polarization difference in terms of a uniform basis of 77 per cent reducing sugars, which is the average percentage of invert sugar after inversion for pure honey. The formulas for making the calculation are then:

$$\text{Per cent pure honey} = \frac{100(P' - P) \times 77}{26.7 \times I} = \frac{288.4(P' - P)}{I}$$

$$\text{Per cent commercial glucose} = 100 - \frac{288.4(P' - P)}{I}$$

in which P' = the Ventzke polarization of the inverted honey at 87° C.
P = the Ventzke polarization of the inverted honey at 20° C.
I = the per cent of invert sugar in the honey after inversion.

Another method, used in European countries, for estimating the amount of commercial glucose in honey is based upon the variation in the invert polarization of the sample from that of pure honey. Calling the average invert polarization of pure honey —17.5 at 20° C. (Table LXX) and employing the figure +175° V. for the polarization of commercial glucose sirup, then if x = per cent of honey in sample, y = per cent of commercial glucose in sample, P = invert polarization of

[14] "Chemical Analysis and Composition of American Honeys," p. 60, *Bull.* 110; U. S. Bur. Chem.

sample in degrees Ventzke, $x + y = 100$.

$$-0.175\,x + 1.75\,y = P$$

$$y = \frac{P + 17.5}{1.93}$$

This method of calculation, the same as that based upon the polarization at 87° C., makes no allowance for the wide range in the invert polarization of individual honeys (—30 to +15), so that a considerable error may be introduced in the final result.

In Table LXXI the polarizations of 5 honeys and of mixtures of the same, with 20 per cent commercial glucose, are given together with the percentage of commercial glucose as calculated by the three methods described.

It will be seen from the results in the table that with admixtures of low-purity honeys and commercial glucose there is a considerable error in the calculation of the percentage of added adulterant. The results obtained by any method for estimating commercial glucose have only an approximate value, and in no case ought such analytical results as those obtained for the pure basswood or white-oak honey to condemn a sample as being adulterated. In all suspicious or doubtful cases confirmatory qualitative tests such as that with iodine should be employed.

TABLE LXXI*

POLARIZATION OF HONEYS AND COMMERCIAL GLUCOSE MIXTURES, WITH CALCULATED PERCENTAGES OF GLUCOSE BY DIFFERENT FORMULAS

Kind of Sample	Direct Polarization, 20° C.	Invert Polarization		Polarization Difference ($P' - P$)	Invert Sugar after Inversion, I	Polarization Difference Corrected to 77 Per Cent Invert Sugar	Calculated Glucose Sirup		
		P 20° C.	P' 87° C.				$\frac{100\,P'}{163}$	$\frac{P + 17.5}{1.93}$	$100 - \frac{288.4(P' - P)}{I}$
	° V.	° V.	° V.	° V.	per cent	° V.	per cent	per cent	per cent
Alfalfa	−19.5	−22.66	+ 3.52	26.18	77.84	25.90	2.16	0.00	3.00
Alfalfa + 20 per cent glucose	+19.4	+16.88	+35.82	18.94	70.01	20.83	21.97	17.82	21.98
Hop vine	−12.6	−16.83	+ 9.68	26.51	75.83	20.92	5.94	.35	.00
Hop vine + 20 per cent glucose	+24.9	+21.54	+40.74	19.20	68.14	21.70	25.00	20.28	18.72
Whitewood	− 4.9	− 9.68	+15.40	25.08	71.88	26.87	9.45	4.06	.00
Whitewood + 20 per cent glucose	+31.1	+27.26	+45.32	18.06	64.99	21.40	27.80	23.25	19.85
Basswood	− .3	− 1.32	+23.21	24.53	70.60	26.75	14.24	8.40	.00
Basswood + 20 per cent glucose	+34.8	+33.94	+51.57	17.63	63.97	21.22	31.64	26.72	20.52
White oak	+11.0	+ 5.17	+28.60	23.43	70.44	25.61	17.56	11.23	4.06
White oak + 20 per cent glucose	+43.8	+39.14	+55.88	16.74	63.84	20.20	34.28	29.35	24.35

* "Chemical Analysis and Composition of American Honeys," *Bull.* 110, p. 61, U. S. Bur. Chem.

Dextrorotation of Inverted Molasses at 87° C. The observations made upon the dextrorotation of inverted honey at 87° C. also pertain to sugar-cane molasses and sirups, but to a much less degree. Eighteen samples of Louisiana sugar-cane molasses, of known purity, examined by Bryan,[15] gave an average direct polarization at 20° C. of +40.6° V., an average invert polarization at 20° C. of —17.8° V. and an average invert polarization at 87° C. of +2.53, the range of the latter being from 0.0 to +4.18, or an equivalent of 0 to 2.5 per cent commercial glucose.

Determination of Fructose by Polarization at low and High Temperatures

Method of Wiley. A second illustration of the methods of high-temperature polarization is afforded by Wiley's[16] method for estimating fructose. In his description of this method Wiley shows that 1 g. of fructose in 100 ml. of solution gives a variation of 0.0357° V. for each 1° C. difference in temperature. The grams of fructose present in 100 ml. of any solution can be calculated, therefore, from the polarizations made at two widely separated temperatures by means of the formula:

$$F = \frac{P' - P}{0.0357(t' - t)}$$

in which F = grams of fructose in 100 ml. of solution.
P' = Ventzke polarization at high temperature t'.
P = Ventzke polarization at low temperature t.

Determination of Fructose in Honey. The method of Wiley has been adopted for this purpose by the Association of Official Agricultural Chemists.[17] The normal weight of honey is transferred with water to a 100-ml. flask, clarified with 5 ml. of alumina cream, diluted to the mark at 20° C., the solution well mixed and filtered. The filtrate is made alkaline to litmus paper by the addition of powdered anhydrous sodium carbonate to complete the mutarotation, and is polarized at 20° C. The same solution is then polarized in a jacketed tube at 87° C. The reading thus obtained is multiplied by 1.0315 to correct for the expansion of the solution due to the heating. The difference between the readings at 20° and at 87° is divided by 67 × 0.0357, or 2.3919. The result is the grams fructose in 26 g. of honey.

[15] *Bull.* 122, U. S. Bur. Chem., p. 182.
[16] Wiley's "Agricultural Analysis," 3, 267, 1897.
[17] "Methods of Analysis, A. O. A. C.," 5th ed., p. 510, 1940.

From this figure the percentage of fructose in the original sample of honey is calculated by multiplying by 100 and dividing by 26.

Example. The normal weight of honey made up to 100 ml. and polarized at 20° C. gave a reading of −14.8° V.; 26 g. of the same honey made up to 100 ml. and polarized at 87° C. gave a reading of +10.50° V., or +10.83° V. when corrected for expansion of the solution. Required the percentage of fructose.

$$F = \frac{100[(10.83 - (-14.8)]}{26 \times 2.3919} = 41.21 \text{ per cent}$$

Observations of other investigators on the change in the polarization of fructose are shown in Table LXXII.

TABLE LXXII

CHANGE OF POLARIZATION OF FRUCTOSE FOR 1° C. CHANGE OF TEMPERATURE

	A	*B*	*C*
Observer	Change in $[\alpha]_D$ of Fructose per 1° C.	Change in Rotation for a Fructose Solution Reading 100° V. per 1° C. $\frac{100\,A}{92.5}$	Change in Rotation for 1 g. Fructose in 100 ml. per 1° C. $\frac{B}{18.692}$
Dubrunfaut[18]	0.62	0.6702	0.03586
Hönig and Jesser[19]	0.68	0.7351	0.03933
Jungfleisch and Grimbert[20]	0.56	0.6054	0.03239
Gubbe[21]	0.63	0.6811	0.03644
Tuchschmid[22]	0.64	0.6919	0.03702
Average	0.626	0.6767	0.03621

The average value 0.0362 is practically identical with that of Wiley.

Another method of determining the variation in the Ventzke polarization of fructose for changes in temperature is by means of Gubbe's equations (p. 473). Since the specific rotation of glucose is not affected by changes in temperature, the results of Table LXVIII are converted into terms of fructose by dividing the values of columns *A* and *C*, and by multiplying those of column *E*, by 2. The variation in po-

[18] *Compt. rend.*, 42, 901 (1856).
[19] *Z. Ver. deut. Zucker-Ind.*, 38, 1028 (1888).
[20] *Compt. rend.*, 107, 390 (1888).
[21] *Z. Ver. deut. Zucker-Ind.*, 34, 1345 (1884); calculated from results for invert sugar.
[22] *J. prakt. Chem.* [2], 2, 235 (1870); calculated from results for invert sugar.

larization of 1 g. of fructose in 100 ml. for 1° C. change in temperature, as thus determined, is 0.036° V.

Jackson and Silsbee[23] confirmed the average factor 0.036, but observed that it varies somewhat with the concentration and also with the details of manipulation. Later determinations with fructose of high purity and with more accurate temperature control give a somewhat lower value. Jackson and Mathews[24] reported the figure 0.03441. Lothrop[25] found a still lower factor, 0.03415, and a comparison with other methods of determining fructose in honey indicated that the value 0.0357 of the Association of Official Agricultural Chemists is too high.

In performing polarizations at high temperatures it is desirable to make the readings as soon as the solution in the tube has reached temperature equilibrium, as indicated by the thermometer placed in the solution and by the disappearance of striations from the field. After the polarization is noted the temperature is again taken and the average thermometer reading used in the calculation. Prolonged heating at high temperatures causes a destruction of fructose. A difficulty is sometimes experienced in obtaining a clear unobscured field of vision when using the hot-water polariscope tube. Too slow a circulation of hot water through the jacket of the tube, with production of currents of unequally heated solution, is the usual cause of the trouble. The hot water should be several degrees above the desired temperature, and the circulation must be rapid enough to prevent loss of heat by radiation.

Limitations of Methods of High-Temperature Polarization. The method of determining invert sugar or fructose by polarization at widely separated temperatures, though giving good results upon dilute solutions of the pure sugars, gives only an approximation for many sugar mixtures. The method is strictly applicable only when the specific rotations of the accompanying sugars are unaffected by changes in temperature; in all other cases there will be a certain error in the determination depending upon the temperature coefficient and the percentage of other sugars present. Although no other sugars are affected to the same extent as fructose, yet it must be remembered that 1.5 g. arabinose, or 3.0 g. galactose, or 7.0 g. maltose, or 9.0 g. lactose, or 50 g. sucrose produces approximately the same alteration in the Ventzke reading with 1° C. variation in temperature as 1 g. of fructose, or 2 g. of invert sugar.

[23] *Bur. Standards Sci. Paper* 519, p. 594, 1926.
[24] *Bur. Standards J. Research,* 8, 403 (1932).
[25] *J. Assoc. Official Agr. Chem.,* 21, 419 (1938).

But notwithstanding this limitation the method of high-temperature polarization has a distinctive value, and, when employed with due caution, will be found of great service in many problems of analysis and research.

DETERMINATION OF SUGARS BY POLARIZATION BEFORE AND AFTER FERMENTATION

By employing pure cultures of specially selected organisms, it is sometimes possible to ferment one or more sugars of a given mixture, and from the variation in polarization thus produced to calculate the percentage of one or more of the members present.

Action of Pure Yeast Cultures upon Different Sugars. The fermentative action of various yeasts upon different sugars has been studied by Tollens and Stone,[26] Hansen,[27] Fischer and Thierfelder,[28] Kluyver,[29] and many others. The results of their experiments show a pronounced selective action on the part of different yeasts. While pure cultures of such well-known yeasts as *Saccharomyces cerevisiæ* or *Saccharomyces Pastorianus* ferment completely *d*-glucose, *d*-fructose, *d*-mannose, *d*-galactose, sucrose, and maltose, these cultures are without action upon *d*-xylose, *l*-arabinose, rhamnose, sorbose, and lactose. A "milk-sugar yeast," employed by Fischer and Thierfelder, fermented lactose and sucrose completely but did not attack maltose. *Torula dattila* and *Schizosaccharomyces Pombe* ferment *d*-glucose, *d*-fructose, *d*-mannose, and sucrose, but not galactose or lactose; maltose is fermented by the latter organism, but not by the former. *Saccharomyces apiculatus* and *Schïzosaccharomyces octosporus* ferment *d*-glucose, *d*-fructose, and *d*-mannose, but because of their lack of invertase do not ferment sucrose; neither do they ferment galactose or lactose.

Method of Fermentation. In carrying out experiments for the separation of sugars by fermentation it is very essential that the culture of a particular yeast be pure. The presence of foreign yeasts, molds, or bacteria may produce changes in sugars which a pure culture would leave unattacked. The solution to be fermented should be sterilized before inoculating.

The most favorable conditions for the action of the yeast are obtained with a solution containing about 10 per cent sugar and kept at a temperature of about 30° C. It is also necessary, in order to secure a rapid and complete fermentation, to have a suitable supply of nu-

[26] *Ann.*, 249, 257 (1888).
[27] *Chem. Zentr.*, 1888, 1208, 1390.
[28] *Ber.*, 27, 2031 (1894).
[29] Kluyver, "Biochemische Suikerbepalingen," p. 19, 1914.

tritive matter present for the growth and sustenance of the yeast. A food supply for yeast in fermentation experiments is generally furnished by means of a nutritive salt solution or by means of yeast extract.

Hayduck's Nutritive Salt Solution. Dissolve 25 g. potassium phosphate, 8 g. crystallized magnesium sulfate, and 20 g. asparagine in 1000 ml. of spring water.

One milliliter of the above solution to each 25 ml. of liquid to be fermented insures a favorable development of yeast.

Yeast Extract. Wash 100 g. of pure yeast (starch-free) repeatedly with cold water and re-press. The residue of yeast is then heated to boiling for one-fourth hour with 500 ml. of water; the liquid is then filtered through a folded filter, the filtrate, if turbid, being returned to the filter until the extract runs through perfectly clear. The extract is then made faintly acid with citric acid, when it is sterilized and preserved in flasks closed by cotton wadding.

The liquid to be fermented is diluted with an equal volume of the above extract.

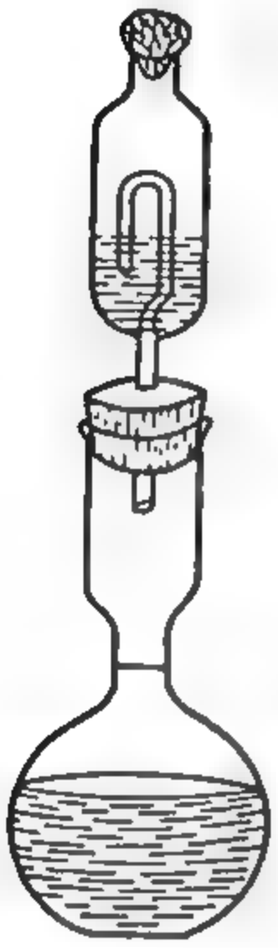


FIG. 207. Fermentation flask.

Fermentation experiments are best carried out in flasks closed with a washing tube for the escape of carbon dioxide. The apparatus shown in Fig. 207 answers very well for the purpose. The fermentation is continued until bubbles of gas cease to pass through the water in the washing tube, when the process is considered to be finished. The washing tube is then removed, the solution heated to expel all carbon dioxide, and, after cooling, clarified, and the volume completed to the mark.

The polarization of the filtered solution is calculated to unfermented sugar, and the difference in polarization, before and after fermentation, calculated to fermented sugar. The application of the method is best understood from a special case.

Example. By hydrolyzing a sample of sawdust with sulfuric acid, treating the resultant liquid with an excess of powdered calcium carbonate, filtering and evaporating, a sirup resulted which contained the two sugars, glucose and xylose.

Fifty grams of the sirup, made up to 100 ml., gave a polarization of +43.5° V. in a 200-mm. tube.

Fifty grams of the sirup was then diluted in a 200-ml. flask with 100 ml. of water and 5 ml. of nutritive salt solution. After sterilizing, cooling, and inoculating with pure-yeast culture, the flask was closed with a washing tube and fermented for 5 days in an incubator at 30° C. The evolution of gas having ceased, the solution was heated to expel CO_2, cooled, clarified with a little normal acetate of lead solution, made up to 200 ml., and filtered. The

polarization of the filtrate in a 400-mm. tube was +5.2° V. Required the percentages of glucose and xylose in the sirup.

The loss in polarization by fermenting was 43.5 − 5.2 = 38.3° V. Since 1° V. = 0.3226 g. glucose in 100 ml. then the grams of glucose fermented were 38.3 × 0.3226 = 12.36 g. or 24.7 per cent glucose (uncorrected) in the sirup.

Since 1° V. = 0.91 g. xylose in 100 ml., then, calling the residual polarization of +5.2 as due entirely to xylose, 5.2 × 0.91 = 4.73 g. or 9.46 per cent xylose (uncorrected) in the sirup.

Corrections for concentration are made as indicated on p. 299.

Determination of Dextrin in Fruit Products. The fermentation method is sometimes employed for the determination of dextrin in jams, jellies, and other products which might be adulterated with commercial glucose. The provisional method of the Association of Official Agricultural Chemists is as follows:[30]

Dissolve 10 g. of the sample in a 100-ml. flask, add 20 mg. of potassium fluoride, and then about one-quarter of a cake of compressed yeast. Allow the fermentation to proceed below 25° C. for 2 or 3 hours to prevent excessive foaming, and then place in an incubator at a temperature of from 27° to 30° C. for 5 days. At the end of that time, clarify with lead subacetate and alumina cream, make up to 100 ml., filter, and polarize in a 200-mm. tube. A pure fruit jelly will show a rotation of not more than a few tenths of a degree either to the right or to the left. If a polariscope having the Ventzke scale is used and a 10 per cent solution is polarized in a 200-mm. tube, the number of degrees read on the sugar scale of the instrument multiplied by 0.8755 will give the percentage of dextrin, or the following formula may be used:

$$\text{Percentage of dextrin} = \frac{C \times 100}{198 \times L \times W}$$

in which C = degrees of circular rotation.
L = length of tube in decimeters.
W = weight of sample in 1 ml.

The factor 0.8755 is found as follows: Calling +198 the $[\alpha]_D$ of dextrin, then the grams of dextrin (D) in 100 ml. of solution are found from the Ventzke reading (V) in a 200-mm. tube by the formula:

$$D = \frac{100(V \times 0.3467)}{2 \times 198} = 0.08755\ V.$$

If 10 g. of product are made up to 100 ml. then the percentage of dextrin in the sample $= \frac{0.08755\ V}{10} \times 100 = 0.8755\ V.$

[30] "Methods of Analysis of the A. O. A. C.," 5th ed., p. 350, 1940.

The use of potassium fluoride in the method just described is to prevent the development of bacteria. Its employment is not necessary when pure-yeast cultures are used and the solution to be fermented has been previously sterilized.

The work of Brown and Morris[31] shows that the dextrins and maltodextrins of starch conversion are not fermented by *Saccharomyces cerevisiæ;* their experiments prove, however, that other yeasts, such as *Saccharomyces ellipsoideus* and *Saccharomyces Pastorianus,* strongly ferment these dextrins. In carrying out the fermentation method for the estimation of dextrin, it is best to work with a pure culture of *Saccharomyces cerevisiæ.*

Limitations of Fermentation Methods. The methods of estimating sugars by difference in polarization, before and after fermentation, give at best only a fair approximation. Several dangers attend the employment of the method, chief among which are the attack of sugars or carbohydrates supposed to be unfermented, and the incomplete destruction of sugars supposed to be completely fermented. Careful attention to the details of pure culture, sterilization, and nutrition will, however, largely eliminate these dangers. The formation of optically active fermentation by-products may introduce a disturbing factor under certain irregular conditions, but with a normal alcoholic fermentation the error from this cause is insignificant. The optical activity of the nutritive solution used in the experiments should of course be determined, and its value, if significant, should be considered in the calculation.

The length of time required for completing a determination has been a strong objection against the use of fermentation methods in general sugar analysis. The more rapid, and generally more accurate, methods based upon polarizing and copper-reducing power have, for this reason, been given the preference.

POLARISCOPIC METHODS BASED ON DESTROYING THE OPTICAL ACTIVITY OF REDUCING SUGARS

The determination of sugars by methods of this class is based upon the fact that solutions of reducing sugars, when heated with alkalies or alkalies and hydrogen peroxide, or with alkalies and metallic oxides or salts, lose their optical activity more or less completely. These methods have been applied not so much to the determination of reducing sugars themselves, as to the determination of sucrose, dextrin, and other non-reducing carbohydrates in presence of reducing sugars.

[31] *J. Chem. Soc. Trans.,* **47, 527 (1885).**

Destruction of Optical Activity of Reducing Sugars by Means of Alkalies

Method of Dubrunfaut. The first efforts to establish a quantitative method in this direction were made by Dubrunfaut[32] in 1850. Later investigators found, however, that the end products in Dubrunfaut's method, obtained by the action of different alkalies upon reducing sugars, were not completely inactive, so that the polariscopic reading always required a certain correction. Efforts to establish a constant correction factor for modifications of Dubrunfaut's method have been made by Pellet,[33] Jesser,[34] Koydl,[35] Bardach and Silberstein,[36] and others, but the results, on account of the variability in conditions, have not been wholly satisfactory.

Method of Lobry de Bruyn and van Ekenstein. The rate of destruction of optical activity upon heating solutions of reducing sugars with dilute alkalies is illustrated by the following experiment taken from the work of Lobry de Bruyn and van Ekenstein;[37] 20 g. of anhydrous glucose was heated with 10 ml. of normal potassium hydroxide in 500 ml. of solution at 63° C. The following decrease in rotation was noted:

Time	Angular Rotation	Specific Rotation	Time	Angular Rotation	Specific Rotation
minutes			minutes		
10	+5° 30′	$[\alpha]_D = +46$	50	1° 50′	
20	4° 20′		85	0° 43′	
30	3° 10′		135	±0° 10′	$[\alpha]_D = \pm 1$
40	2° 20′				

At the end of the experiment the solution had not darkened perceptibly and the original reducing power had only slightly diminished.

Explanation of Optical Inactivity Produced by Alkalies. The explanation of the change of an optically active into an optically inactive solution of reducing sugar by action of alkalies was first given by Lobry de Bruyn and van Ekenstein. In the experiment just quoted the optical inactivity of the solution is due not to a destruction of glucose,

[32] *Compt. rend.*, 32, 439 (1851).
[33] *Bull. assoc. chim. sucr. dist.*, 8, 623 (1890/91).
[34] *Oesterr. ungar. Z. Zuckerind. Landw.*, 27, 35 (1898).
[35] *Oesterr. ungar. Z. Zuckerind. Landw.*, 29, 381 (1900).
[36] *Z. Untersuch. Nahr. u. Genussm.*, 21, 540 (1911).
[37] *Rec. trav. chim.*, 14, 156, 203 (1895); 16, 262 (1897).

but to its partial conversion into mannose and fructose, the combined rotations of the mixture of sugars producing optical neutrality. In one experiment the authorities just named noted after heating with alkali a loss of 18 per cent in reducing power; the residue was estimated to consist of 49 per cent unchanged glucose, 5 per cent mannose, and 28 per cent fructose; the calculated rotation of such a mixture would in fact be very nearly zero.

Method of Jolles. Experiments by Jolles[38] upon arabinose, glucose, fructose, invert sugar, lactose, and maltose show that these sugars in 1 to 2 per cent solution are rendered optically inactive by heating for 24 hours at 37° C. with 0.01 normal sodium hydroxide while sucrose is completely unchanged by this treatment. Stronger solutions of reducing sugars than 2 per cent show usually a residual activity after the alkaline treatment; it is necessary, therefore, in Jolles's method to dilute solutions to 2 per cent reducing sugar before making the determination. With substances containing much reducing sugar such dilution necessarily involves a considerable multiplication of any errors in the polariscope reading.

Method of Bardach and Silberstein. Bardach and Silberstein[39] have modified Jolles's method so as to include solutions of reducing sugar up to 5 per cent concentration. Their method of procedure is as follows:

Take 45 ml. of the neutralized sugar solution and make up to 50 ml. with normal sodium hydroxide, thus making the solution 0.1 normal alkaline. The solution is then polarized and a measured volume placed in a small beaker (8 to 10 cm. high and 5 cm. in diameter) and kept at 36° to 39° C. for 20 hours by means of a thermostat, the beaker remaining uncovered. The solution is then cooled, made up to the original volume, and repolarized. The final polarization is corrected for residual activity by means of an empirical factor, which for glucose was found to be as shown in Table LXXIII.

The loss in polarization, after treatment with alkali under the prescribed conditions, must be diminished, therefore, by about 0.25 to give the correct polarization value of glucose. So also the residual polarization must be increased by 0.25 to give the correct polarization equivalent of the residual sucrose or other non-reducing carbohydrate present.

It is evident that the chemist in employing such methods as the above must establish his own correction factor for the particular reducing sugar with which he is working. The lack of absolute uni-

[38] *Z. Untersuch. Nahr. u. Genussm.*, **20, 631 (1910).**
[39] *Loc. cit.*

formity of conditions in the analysis of impure sugar products leaves the general reliability of such correction factors more or less in doubt.

TABLE LXXIII

Change in Polarization of Glucose upon Warming with Dilute Alkali

Approximate Percentage of Glucose in Solution	Polarization Value		Approximate Percentage of Glucose in Solution	Polarization Value	
	Before Treatment	After Treatment		Before Treatment	After Treatment
0.5	+0.51	−0.09	2.5	+2.54	−0.36
1	+1.02	−0.19	3	+3.05	−0.26
1	+1.02	−0.15	3	+3.06	−0.27
1.5	+1.53	−0.26	4	+4.10	−0.32
2	+2.04	−0.25	4	+4.07	−0.25
2	+2.05	−0.26	5	+5.12	−0.21

Haddon's Baryta Method.[40] Certain cane varieties, such as the Uba cane grown in South Africa, contain varying amounts of starch which passes into the juice and appears in the molasses in the form of hydrolytic products of high rotation. With such products the Clerget acid method does not give correct results for sucrose, although inversion with invertase does. Haddon has found that the interfering substances can be eliminated and at the same time the reducing sugars destroyed by boiling with barium hydroxide solution. A solution containing 40 g. molasses in 200 ml. total volume is prepared. Of this solution, 121 ml. is pipetted into a flask, 2 g. of barium hydroxide is added, and the mixture is boiled under reflux for 20 minutes. It is then cooled, washed into a 200-ml. flask, neutralized with acetic acid, and made to the mark. It is clarified with the necessary quantity of Horne's dry lead subacetate, and filtered. To 100 ml. of the filtrate, in a 100–110-ml. flask, 5 ml. of diluted ammonium hydroxide (1 + 1) is added, the volume is made up to the 110-ml. mark, and the solution is again filtered. Fifty milliliters of the filtrate is neutralized, in a 50–55-ml. flask, with glacial acetic acid; 2.5 ml. more of the acid is added; and the volume is completed with water to 55 ml. This solution, containing 10 g. of the original molasses in 100 ml., is polarized in a 200-ml. tube, and the result, in degrees Ventzke, is multiplied by 2.6 to convert it into per cent sucrose. According to Haddon, a sucrose determination in the filtrate by the Clerget method should, and

[40] *S. African Sugar J.*, 13, 833 (1929); *Bull. assoc. chim.* 53, 785 (1936).

usually does, give the same result as the direct polarization. A further study of this method appears necessary before it can be accepted.[41]

Destruction of Optical Activity of Reducing Sugars by Means of Alkali and Hydrogen Peroxide

Other chemicals have been used in connection with alkalies to promote the destruction of reducing sugars. Lemeland,[42] for example, has devised a method for destroying the optical activity of reducing sugars in the presence of sucrose by means of alkali, manganese dioxide, and hydrogen peroxide.

Method of Pellet and Lemeland. Pellet and Lemeland[43] have proposed a method for the analysis of sugar-cane molasses which is based upon destroying the optical activity of reducing sugars by means of alkali and hydrogen peroxide. The details of the method are as follows:

Make a solution of the cane molasses that will contain at most 5 per cent of reducing sugars. Measure 50 ml. of this solution into a 300-ml. flask, add 7.5 ml. of sodium hydroxide (36° Bé.), then 75 ml. of hydrogen peroxide (12 vols.), and 60 ml. of water. Mix, place the flask in a boiling-water bath for 20 minutes, cool, neutralize the remaining alkalinity fairly exactly with acetic acid, and defecate with basic lead acetate solution (36° Bé.), the necessary amount of which will be found to vary from 15 to 40 ml., according to the weight of the material taken, the amount of reducing sugars destroyed, and the impurities initially contained in the liquid. Complete the volume to 300-ml., mix well, and filter. First polarize directly in the 200-mm. or 400-mm. tube. Then 50 ml. of the filtered liquid may be taken, 1 ml. of glacial acetic acid added to it, the volume completed to 55 ml., and after mixing a second polarization made, account being taken of the dilution. This is done because the second polarization is often a little different from the first, in which the liquid is alkaline. If a difference is observed, then the second, or acid polarization, should be used. The percentage of sucrose is calculated on the solution, and then on the sample.

The authors state that the results by this method agree very closely with those obtained by the method of inversion, when special precautions are observed to insure the utmost accuracy, but Cross and

[41] The destruction of reducing sugars by alkali has been used by Fincke for the determination of sucrose and lactose in milk chocolate, by determining the polarization before and after the destruction of lactose by heating with calcium hydroxide (*Z. Untersuch. Lebensm.*, **50**, 351 [1925]).

[42] *J. Pharm. Chim.*, **2**, 298 (1910).

[43] *Intern. Sugar J.*, **13**, 616 (1911).

Taggart[44] found that the method does not give correct results even with mixtures of pure sugars. Reduction of the time of heating did not improve matters, but fairly good results were obtained when the temperature was reduced from near 100° C. to 55° C. Subsequently, Schneller[45] made a careful study of the method and reached the conclusion that there are three sources of error:

(1) The residuary levorotation. This introduces a considerable error, especially with material high in reducing sugar and with methods using a weak concentration of alkali.

(2) Stronger concentration of alkali reduces this levorotation, but introduces a second serious error, due to the decrease of sucrose rotation by the resulting alkali salts.

(3) Oxidation of sucrose in alkaline solution, especially in the hydrogen peroxide methods.

Apparently correct results may be obtained by a compensation of errors. Schneller experimented with hydrosulfite and sulfur dioxide, as a decolorant and neutralizing agent, instead of the hydrogen peroxide and acetic acid, but the results were not any better.

Destruction of Optical Activity of Reducing Sugars by Means of an Alkaline Lead Solution

Method of Schneller. Lobry de Bruyn and van Ekenstein had found that lead hydroxide and lead subacetate have an effect on reducing sugars similar to that of alkalies and alkaline earths. Schneller, repeating this work, observed that when invert sugar is heated with subacetate of lead there is a slight residual positive reading. With a proper combination of alkali and of lead acetate it was to be expected that the effects of the two reagents would be mutually compensating. This was indeed found to be so; but the two reagents must be added simultaneously and not one after the other; in the latter case a residual levorotation is obtained. On this basis Schneller worked out the following method, primarily for the determination of sucrose in invert-sugar sirups:[46]

A solution of 20 g. of lead acetate is gradually poured into a solution of 65 g. sodium hydroxide in a liter flask. The solution is heated, if necessary, until it becomes clear, and after cooling made up to 1 liter. The normal sugar weight of the sirup is transferred with about 100 ml. of water into a 200-ml. flask. In the case of completely inverted

[44] *Louisiana Bulletin*, 135, 47 (1912).
[45] *Louisiana Bulletin*, 156 (1916).
[46] Private communication.

sirups, 20 ml., and for semi-inverted sirups about 14 ml., of the above lead reagent is added. The flask is heated for about 30 minutes in a boiling-water bath, and after cooling to room temperature it is neutralized with 2 or 3 ml., respectively, of 50 per cent acetic acid. (This addition of acetic acid is not absolutely necessary except with sirups containing very little invert sugar, or with raw sugars; but the remaining alkalinity is so strong in this case that lead saccharate may be precipitated. It is safer always to add the acid, although a darker solution is obtained.) For clarification about 15 ml. of lead subacetate solution is used, the volume is made up to 200 ml., and the solution filtered. Fifty milliliters of the filtrate is made up to 55 ml. with 50 per cent acetic acid in a 50–55-ml. flask, and the solution is polarized. The reading is multiplied by 2.2 to obtain the per cent sucrose.

The method checks with the invertase method for solutions containing sucrose and invert sugar, but low results are usually obtained on low-purity products like molasses or refinery sirups.

Destruction of Optical Activity of Reducing Sugars by Means of an Alkaline Bismuth Solution

Muller's Method.[47] This is based on the destruction of reducing sugars by the use of a solution similar to Nylander's reagent (see p. 650). Twenty-five grams Rochelle salts and 32 g. sodium hydroxide are dissolved in 400 ml. of water under the application of heat; 11 g. of bismuth subnitrate is gradually added, and the heating is continued until it is all dissolved. The solution is cooled, made up to 500 ml., and filtered.

For the analysis, 20 g. of molasses is dissolved in 40 ml. boiling water and transferred to a 300-ml. flask; 15 ml. of the bismuth reagent is added, and the mixture is heated on a boiling-water bath for 15 minutes. After cooling, 150 ml. of cold water and the equivalent of 60 ml. of basic lead acetate solution of 36° Baumé (French Pharmacopeia) are added, the volume is completed to the mark, and the contents of the flask are shaken and filtered. One hundred milliliters of the filtrate is introduced into a 100–110-ml. flask, 5 ml. of acetic acid and sufficient water to the upper mark are added, the contents treated with 2 g. decolorizing carbon, mixed, and filtered. The reading is taken in a 400-mm. tube. If a saccharimeter with a 20-g. normal sugar weight is used, the reading is multiplied by $3 \times 1.1 \div 2$, or 1.65, to obtain the percentage of sucrose. If an instrument with a different normal weight is employed, the reading must be corrected accordingly. The

[47] *Intern. Sugar J.*, **18, 274 (1916).**

15 ml. of bismuth reagent is sufficient to destroy 3 g., or 15 per cent of reducing sugars; if the sample contains more than this, an additional milliliter of reagent must be used for each per cent reducing sugars above 15. The results obtained checked closely with those of Pellet's sulfurous acid method of double polarization (see p. 446). According to Muller the method is applicable to all the products of the cane-sugar factory, but not to beet products, which contain considerable quantities of amino compounds that are not destroyed by the reagent used.

Destruction of Optical Activity of Reducing Sugars by Means of Alkali and Mercuric Cyanide

Method of Wiley. The destruction of the optical activity of reducing sugars by means of Knapp's alkali mercuric cyanide solution was first employed by Wiley[48] in the determination of dextrin in commercial glucose. The reagent is prepared as follows:

Alkali Mercuric Cyanide Solution. Dissolve 120 g. sodium hydroxide and 120 g. mercuric cyanide in separate portions of water; the two solutions are then mixed and made up to 1000 ml. Any precipitate which forms is removed by filtration.

In making the determination 10 g. of the commercial glucose is dissolved in water and made up to 100 ml.; 10 ml. of this solution is transferred to a 50-ml. graduated flask, 20 to 25 ml. of the alkali mercuric cyanide solution is added, and the mixture is boiled 3 minutes under a well-ventilated hood. The solution is cooled, and neutralized with concentrated hydrochloric acid, the acid being added until the brown color of the liquid is just discharged. The solution is then clarified, made up to volume, filtered, and polarized. The optical activity of the maltose and dextrose being destroyed, the residual polarization is that of the dextrin.

In Wiley's experiments, the specific rotation of the dextrin was taken as +193. Adopting this figure, and taking the reading on a Ventzke-scale saccharimeter, the grams of dextrin in 100 ml. of solution $= \frac{66.5 \times 0.26}{193} V^\circ = 0.0896\ V^\circ$. Since the solution polarized contained 1 g. of original sample in 50 ml. (or 2 g. in 100 ml.), then $\frac{0.0896\ V^\circ}{2} \times 100$ = per cent dextrin in the commercial glucose.

In concluding this chapter upon special methods of saccharimetry the chemist is advised, as in the methods of inversion, to test the re-

[48] Wiley's "Agricultural Analysis," 3, 290, 1897.

liability of any untried process by means of check analyses upon mixtures of known composition. In sucrose determinations, parallel analyses should be made by the invertase method. It is only in this way that an idea can be formed of the errors which are due to defect of method or to personal equation.

CHAPTER XII

MISCELLANEOUS PHYSICAL METHODS AS APPLIED TO THE EXAMINATION OF SUGARS AND SUGAR SOLUTIONS

In addition to specific gravity, refractive index, and specific rotation there are a number of other physical constants or properties which, though of lesser analytical importance, have nevertheless a considerable value in certain investigations of sugars and sugar solutions. Among the properties of this class may be mentioned viscosity, heat of combustion, osmotic pressure, rate of diffusion, surface tension, heat of solution, thermal and electrical conductivity, hydrogen-ion concentration, specific heat, magnetic rotation, color, and turbidity. It is beyond the scope of the present treatise to discuss the methods of making each one of these physical measurements. However, some of these properties have become of considerable importance in general laboratory practice, and the present chapter will discuss their use in the investigation of sugars and sugar products. Physical methods which are of more special application will be taken up in Chapter XVII.

VISCOSITY OF SUGAR SOLUTIONS

The determination of viscosity is a measurement which is frequently applied to solutions of sugars and other carbohydrates for special purposes of technology, analysis, or research.

Viscosity is defined as the tangential force which a fluid flowing in one plane exerts on an adjacent plane. Viscosity is unity when a force of 1 dyne acts between two adjacent layers of fluid per unit of area, and the space rate of variation of the tangential velocity from layer to layer is unity. This unit of viscosity is the "poise," named after Poiseuille. The viscosity of water at 20° C. equals about 1 centipoise (1/100 of a poise), while that of a 60 per cent cane-sugar solution at 10° C. is a little over 1 poise.

There are many different methods and instruments for determining viscosity, and only a few typical examples will be described, particularly those which have actually been used and tested in fundamental or technical research on sugar products.[1]

[1] For a detailed discussion of viscosimetry see Hatschek, "The Viscosity of Liquids," London, 1928.

Capillary Viscosimeters; Law of Poiseuille. The capillary method is based on the law of Poiseuille. If V is the volume of liquid discharged in a time t through a capillary tube of length l and radius r, under the pressure p, then

$$V = \frac{\pi \times p \times r^4 \times t}{8\eta \times l}$$

where η is the viscosity of the liquid. It follows from the foregoing that

$$\eta = \frac{\pi p r^4 l}{8\,Vl}$$

The ideal conditions under which the Poiseuille formula holds are difficult to realize experimentally, and for this reason it is necessary in the determination of absolute viscosities to apply various corrections. But in practice it is usually sufficient to calibrate any given viscosimeter with liquids of known absolute viscosity. When V, l, and r are unchanged, as happens in the use of the same viscosity apparatus, η under constant pressure becomes equal to Kt, in which K is a single constant peculiar to each individual viscosimeter.

If the liquid is permitted to flow through the capillary downward under the effect of gravity, as in many of the instruments in common use, the pressure p equals $h \times d \times g$, where h is the mean height, which for the same viscosimeter is constant, d is the density of the liquid, and g the gravitation constant. It follows that with this type of viscosimeter

$$\eta = Cdt$$

where C is again a constant peculiar to the viscosimeter used. This equation may also be written

$$\frac{\eta}{d} = Ct$$

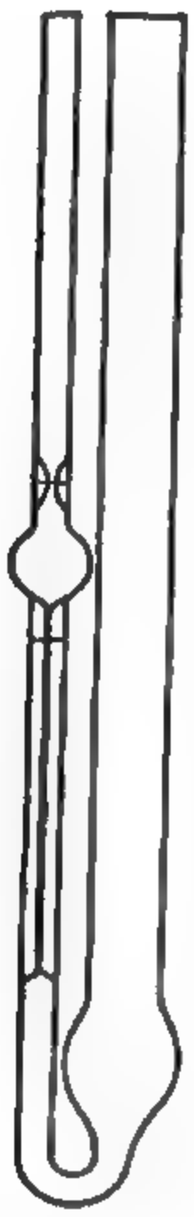
It is seen that under these conditions the time of outflow t is not a direct measure of η, but of η/d. This quotient, also designated by the Greek letter ν, is termed " kinematic " viscosity, as distinguished from the absolute or " dynamic " viscosity η. The unit of kinematic viscosity is the " stokes," equal to 1 poise per unit density.

Viscosity Pipette. The simplest viscosimeter is an ordinary pipette with two marks and narrow tip, Fig. 208.

The pipette is first filled with water so that its meniscus coincides with the upper mark A; after being held in a perfectly upright position the water is released and the interval of time for the passage of the

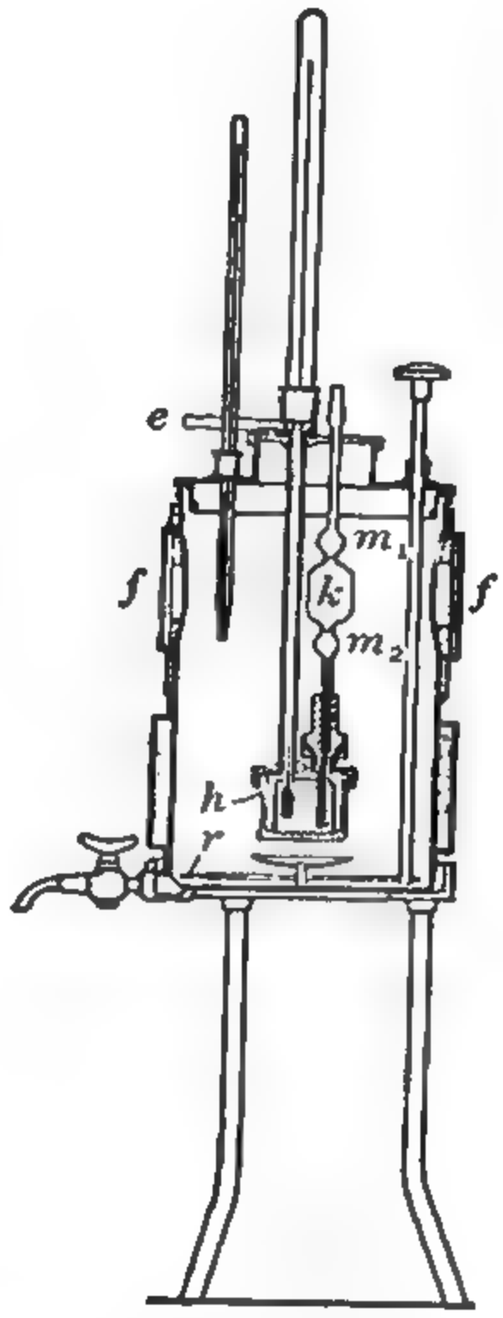
meniscus from *A* to the lower mark *B* is noted. The process is repeated a number of times and the average result taken as the water constant of the pipette at the temperature of the experiment. The pipette is dried and the process repeated in exactly the same manner with a sugar solution. If the average time of flow at 20° C. for water

FIG. 208. Viscosity pipette.



(*Courtesy of Eimer and Amend.*)

FIG. 209. Ostwald's viscosity pipette.



(*Reproduced from Erk, "Zähigkeits-messungen," p. 46.*)

FIG. 210. Vogel-Ossag viscosimeter.

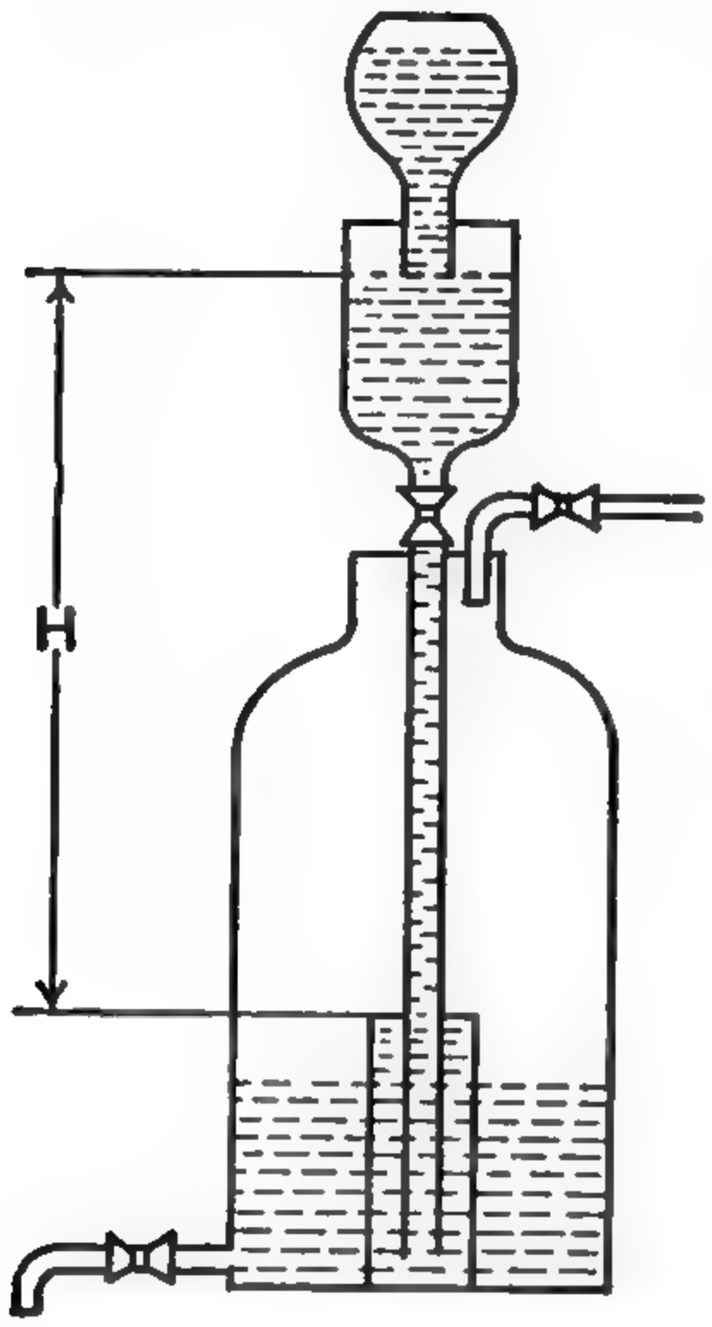
is 20.2 seconds and that of a sugar solution at 20° C. 105.1 seconds, then $105.1/20.2 = 5.2$ is the relative kinematic viscosity of the sugar solution at 20° C., as compared with water of the same temperature.

The viscosity pipette designed by Ostwald is shown in Fig. 209. It has the advantages that measurements on the same solution can be repeated in quick succession and that it may be immersed in a thermostat.

Vogel-Ossag Viscosimeter. Greater precision may be obtained with the Vogel-Ossag technical viscosimeter, a modified form of which has been used by Spengler and Landt[2] for viscosity determinations on sugar products. As may be seen from Fig. 210, it represents an improved Ostwald viscosimeter. It is provided with several interchangeable capillaries, each one of which must be calibrated with a

[2] *Z. Ver. deut. Zucker-Ind.*, **80, 523 (1930).**

suitable liquid of known viscosity. The lower end of the pipette dips into a double-walled chamber. This makes it possible to carry out measurements at varying, increasing temperatures without opening the apparatus.



(Reproduced with permission from Z. Ver. deut. Zucker-Ind. 80, 532.)

FIG. 211. Apparatus of Spengler and Landt for applying constant excess pressure in viscosity determinations.

This instrument may be used for measuring either kinematic viscosity or dynamic viscosity directly. In the former case the liquid is drawn by suction into the pipette and allowed to flow out by gravity; the time during which the surface of the liquid moves from the upper to the lower mark is determined, and the viscosity is found by comparison with the time of outflow of a standard liquid of known kinematic viscosity. This mode of procedure has the disadvantage that, according to the viscosity of the liquid being examined, larger or smaller quantities adhere to the wall of the pipette bulb, the volume of the liquid being affected thereby.

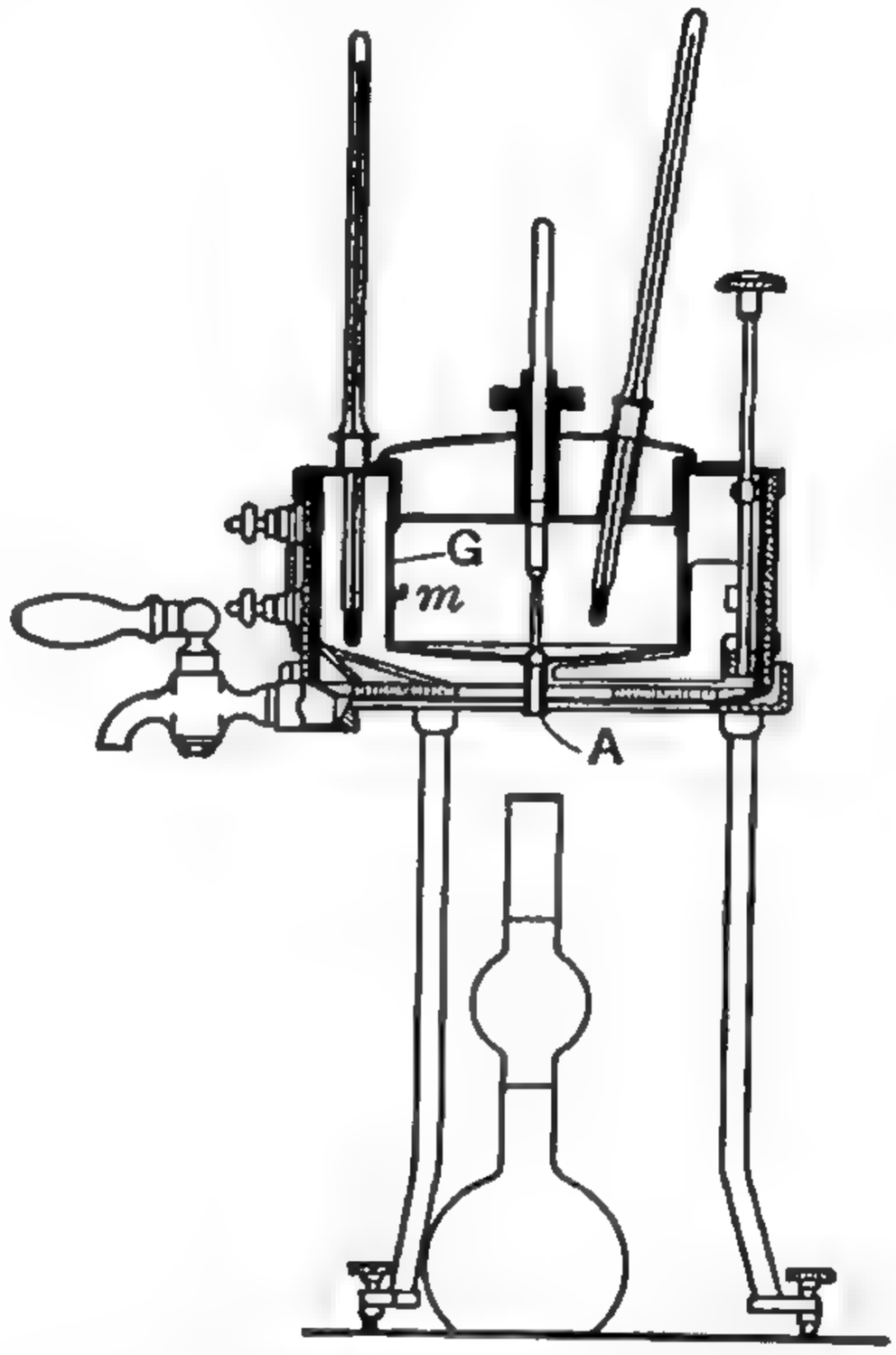
It is therefore better to determine the dynamic viscosity directly by forcing the liquid into the pipette from below, applying constant excess pressure at *e*. A convenient apparatus for supplying the excess pressure, used by Spengler and Landt, is illustrated in Fig. 211. The dimensions are chosen so that the excess pressure ($P = H$) amounts to 60 to 76 cm. of water. The dynamic viscosity is calculated directly from the times of flow for the liquid under examination and that of the liquid of known dynamic viscosity. When this mode of operation is employed, the density of the two liquids must be approximately the same, or else the pressure P_1 actually applied to the liquid of density d_1 must be calculated from the equation (see p. 498)

$$P_1 - (hd_1g) = P - (hdg)$$

Ubbelohde[3] has shown that in the usual capillary viscosimeters the effective height of the liquid varies with the surface tension. More accurate results may be obtained with a pipette constructed on the principle of the "suspended level." Ubbelohde's pipette is similar to Ostwald's in appearance, but the lower end of the capillary widens

[3] *J. Inst. Petroleum Tech.*, 19, 376 (1933); *Ind. Eng. Chem., Anal. Ed.*, 9, 85 (1937).

into a cylindrical bulb which has a vertical side arm, opening to the air, attached to it. The liquid upon emergence from the lower end of the capillary flows in a thin layer along the wall of the bulb, and a hemispherical suspended level forms at the tip of the capillary. This suspended level is always at the same location independent of the kinematic viscosity, specific gravity, and other properties of the liquid. The hemispherical suspended level causes an amount of traction which exactly counterbalances the effect of the surface tension on the upper meniscus of the liquid. The height of the liquid becomes constant, and the kinematic viscosity of any liquid can be determined with great precision and accuracy after the apparatus has once been calibrated with a liquid of known viscosity. The only correction to be applied is that for energy of flow; this correction is found from tables given by Ubbelohde. Modifications of the pipette are used for the determination of dynamic viscosity, or of dynamic or static surface tension.



(*Reproduced from Erk, "Zähigkeitsmessungen," p. 32.*)

FIG. 212. Engler's viscosimeter.

Engler's Viscosimeter. The apparatus of Engler (Fig. 212) is very generally used for technical purposes in Europe, and to some extent in America. The instrument consists of an outer bath which is filled with water or oil, and heated to the desired temperature either electrically or by a gas burner. The inner container, for the liquid whose viscosity is to be measured, is gold plated, and has an internal diameter of 106 mm. The segment-shaped bottom terminates in a narrow tube *A*, 20 mm. long, 2.9 mm. in internal diameter at its upper end, and 2.8 mm. at its lower end; it can be closed by means of the central valve rod which passes through both covers. The outlet tube has a short nipple, protruding below the bottom of the inner vessel; this nipple is 3 mm. high by 4.5 mm. wide. The inner container holds at the mark *m* exactly 240 ml. of solution. Both the outer and the inner vessel are provided with thermometers. After the inner vessel is filled to the mark *m* with water or solution, the cover is placed in position and the temperature brought to the desired point.

The valve rod is then withdrawn and the time noted for the delivery of exactly 200 ml. of liquid into the flask below.

The results obtained with this instrument are usually expressed in terms of the "Engler degree," which is the time of outflow, in seconds, for 200 ml. of water at 20° C. As the liquid flows out under its own weight, the Engler degree, E, of a liquid is a measure of its kinematic viscosity, but is not directly proportional to it. According to Vogel, the relation between Engler degrees and kinematic viscosity may be expressed by the formula:[4]

$$\nu = E \times 0.0760^{(1-E^{-3})}$$

The kinematic viscosity is converted into the dynamic viscosity by multiplying by the density of the liquid.

Falling-Body Viscosimeters; Stokes's Law. The viscosimeters operating on this principle consist usually of a vertical tube, commonly of glass, and a spherical or cylindrical body, of metal or some other substance, which is dropped into the tube containing the liquid. The time necessary for the falling body to pass from an upper to a lower mark is a measure of the viscosity of the liquid in the tube, according to Stokes's law. If a spherical body is used, this law may be written:

$$\eta = \frac{2\,r^2 g\,(D - d)}{9 \cdot v}$$

where r is the radius of the falling sphere, g the gravity constant, D the density of the sphere, d that of the liquid, and v the velocity of the sphere. For v we may substitute L/t, where t is the time required for the body to fall through the distance L. This law holds strictly only when the space occupied by the liquid is infinite. But the correction to be applied for finite dimensions is independent of the viscosity, as has been shown by Ladenburg. Since r, g, and L are constant for any particular apparatus at a given temperature, the above equation may be written

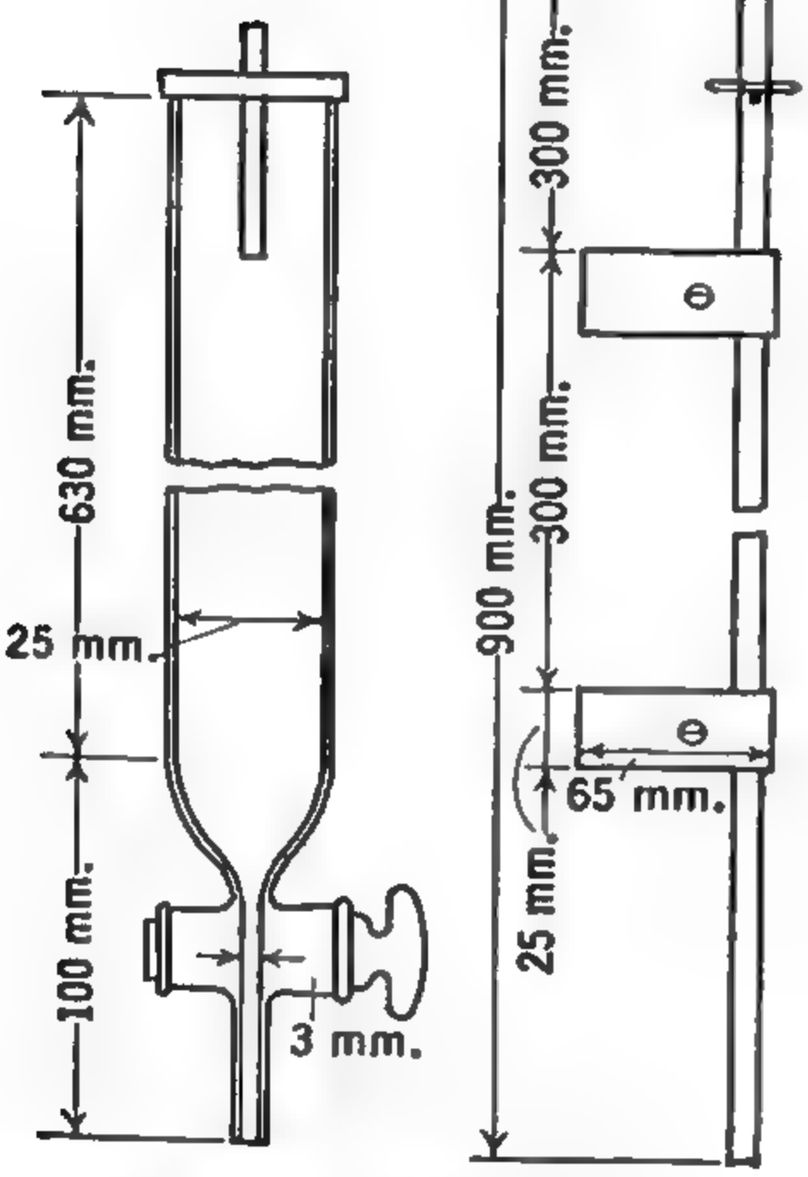
$$\eta = C(D - d)\,t$$

Constant C is determined with a liquid of known viscosity. The variations of r and L with temperature are small so that their effect on the result over a considerable temperature range can usually be neglected.

[4] A diagram by which Engler degrees and readings obtained with other technical viscosimeters may be converted into kinematic viscosity is shown in "International Critical Tables," Vol. I, p. 33.

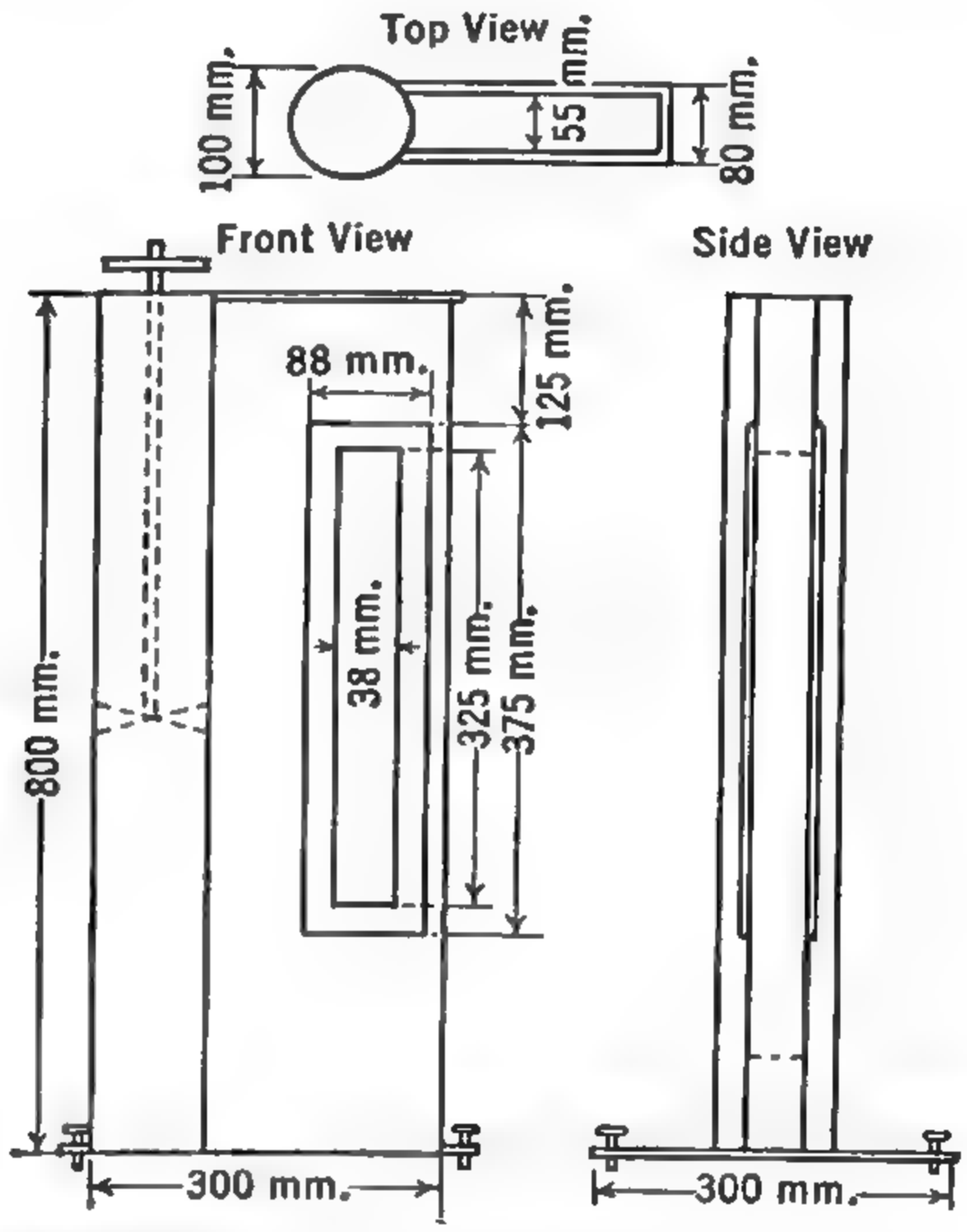
A simple viscosimeter of the falling-sphere type has been described by Bennett and Nees[5] as follows:

The tube and its support are shown in Fig. 213. It is approximately 2.35 cm. in diameter and 65 cm. long, and has a stopcock on the lower end for the purpose of withdrawing the balls after a determination has been made. The distance between the timing marks is 30 cm. A brass cap, properly grooved, fits snugly over the upper end of the tube. Accurately centered in this cap is a short piece of thick-walled glass tube, 0.25 cm. in diameter and 7 cm. long. When the cap is in place this small tube extends about 4.5 cm. into the viscosimeter tube. It serves the dual purpose of freeing the sphere from air bubbles and of insuring its falling through the center of the column of solution.



(Reproduced with permission from Ind. Eng. Chem., 22, 92.)

FIG. 213. Viscosimeter of Bennett and Nees; viscosity tube and support.



(Reproduced with permission from Ind. Eng. Chem., 22, 92.)

FIG. 214. Constant-temperature bath for viscosimeter of Bennett and Nees.

The water bath is shown diagrammatically in Fig. 214, which gives the dimensions of the bath proper. It rests on three leveling screws which, with the aid of a plummet attached to the side of the bath, make it possible to keep the bath and viscosimeter tube in a vertical position. In the bottom of the bath is a socket in which the end of the tube support rests. This support carries, near the top, an arm

[5] *Ind. Eng. Chem.*, **22**, 91 (1930).

with two small lugs which engage two holes on opposite sides of the upper rim of the bath. By this arrangement the tube is held in a rigid, vertical position. The bath is provided with a motor-driven propeller which insures proper circulation of the water, with an electrical heating unit, and with a special mercury thermometer for controlling the temperature. There are two glass windows on opposite sides of the bath for observing the fall of the sphere through the solution under investigation. The bath holds two viscosimeter tubes, so that two solutions can be brought to the desired temperature at the same time.

To carry out a determination, the tube is filled with the liquid, at the desired temperature, to within about 3.5 cm. of the top and is covered with the cap and guide tube, the latter extending about 1 cm. below the surface of the liquid. By moving the cap to one side, the liquid is overlaid with a suitable oil, such as castor oil, to prevent evaporation. As no oil gets into the guide tube, the ball comes in contact only with the solution to be tested. The upper end of the guide tube is closed with a small piece of rubber tubing except when the determination is actually being made. The solution is allowed to stand for a sufficient time to attain uniform temperature and to be completely freed of air bubbles.

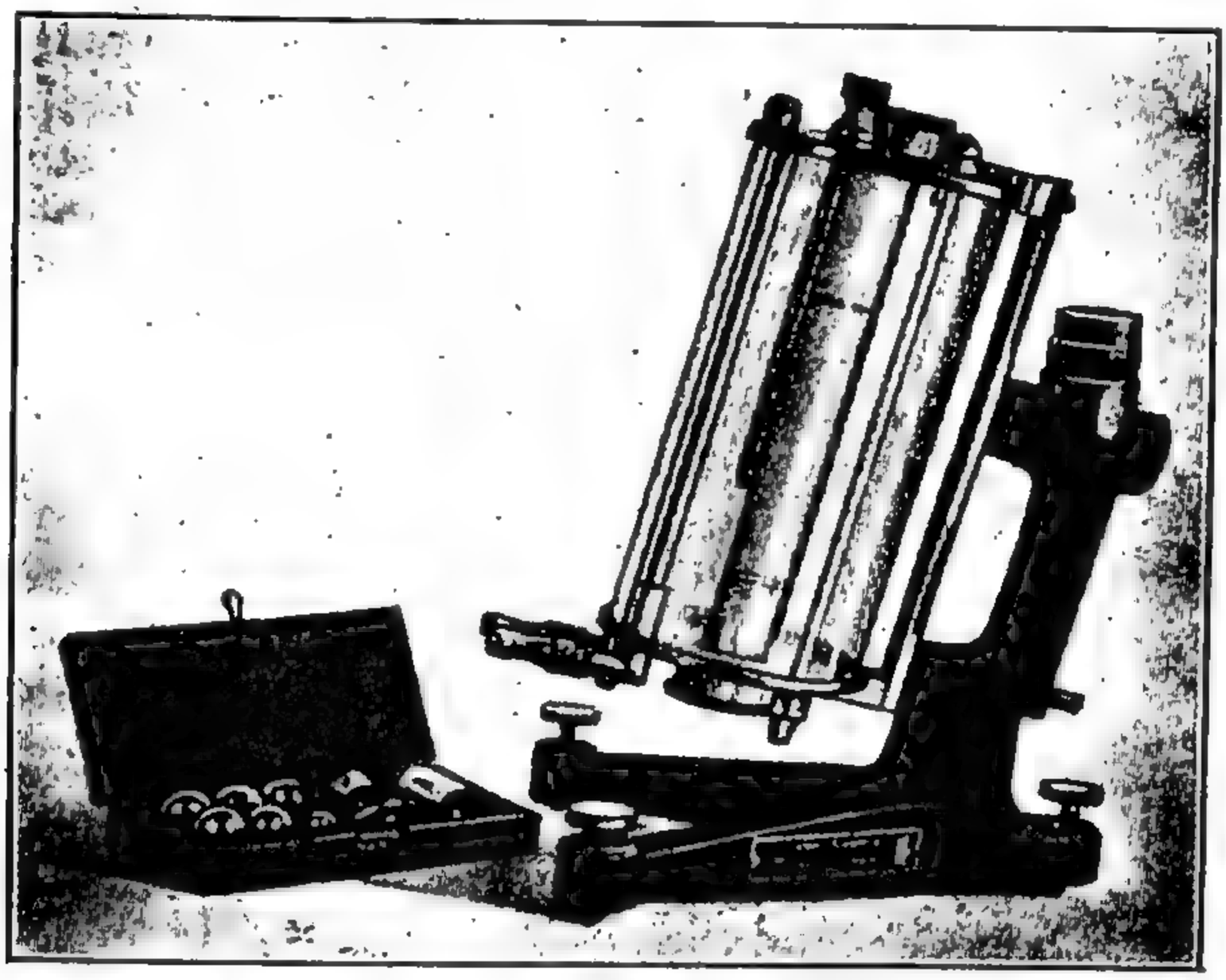
When the solution is in the proper condition a ball is introduced into the guide tube and is timed as it falls between the two marks on the viscosity tube. Aluminum balls may be used for determining the lower viscosities, and steel balls for the higher ones. For the steel balls, ordinary $\frac{1}{16}$-inch ball bearings are suitable. There is always some variation in the dimensions of the balls, and for this reason it is advisable to time several balls, from 5 to 10 according to the variation, and to take the average time noted for calculating the viscosity. Several balls may be in the solution at one time, but they should be at least 5 cm. apart. It is usually necessary to push each ball beneath the surface of the solution in the guide tube. When working with dark solutions, an electric light is placed behind the window in the bath in order that the balls may be plainly seen.

Turbid solutions must first be filtered. The density of the balls and of the solutions is determined with the pycnometer.

The Höppler Viscosimeter. In the apparatus of Bennett and Nees, and in others of the same type, it is presupposed that the ball falls perpendicularly without any turning motion. This condition is not always fulfilled, however, and variable results may be obtained for this reason. In the Höppler viscosimeter[6] this uncertainty is removed

[6] Knop, *Z. Ver. deut. Zucker-Ind.*, **83, 932 (1933).**

by forcing the ball to fall eccentrically along an inclined wall. Höppler found experimentally that the most favorable angle is about 10° from the perpendicular, and that under this condition the dynamic viscosity is directly proportional to the time and to the difference between the densities of the ball and of the liquid, as expressed by the simplified Stokes formula given on p. 502.

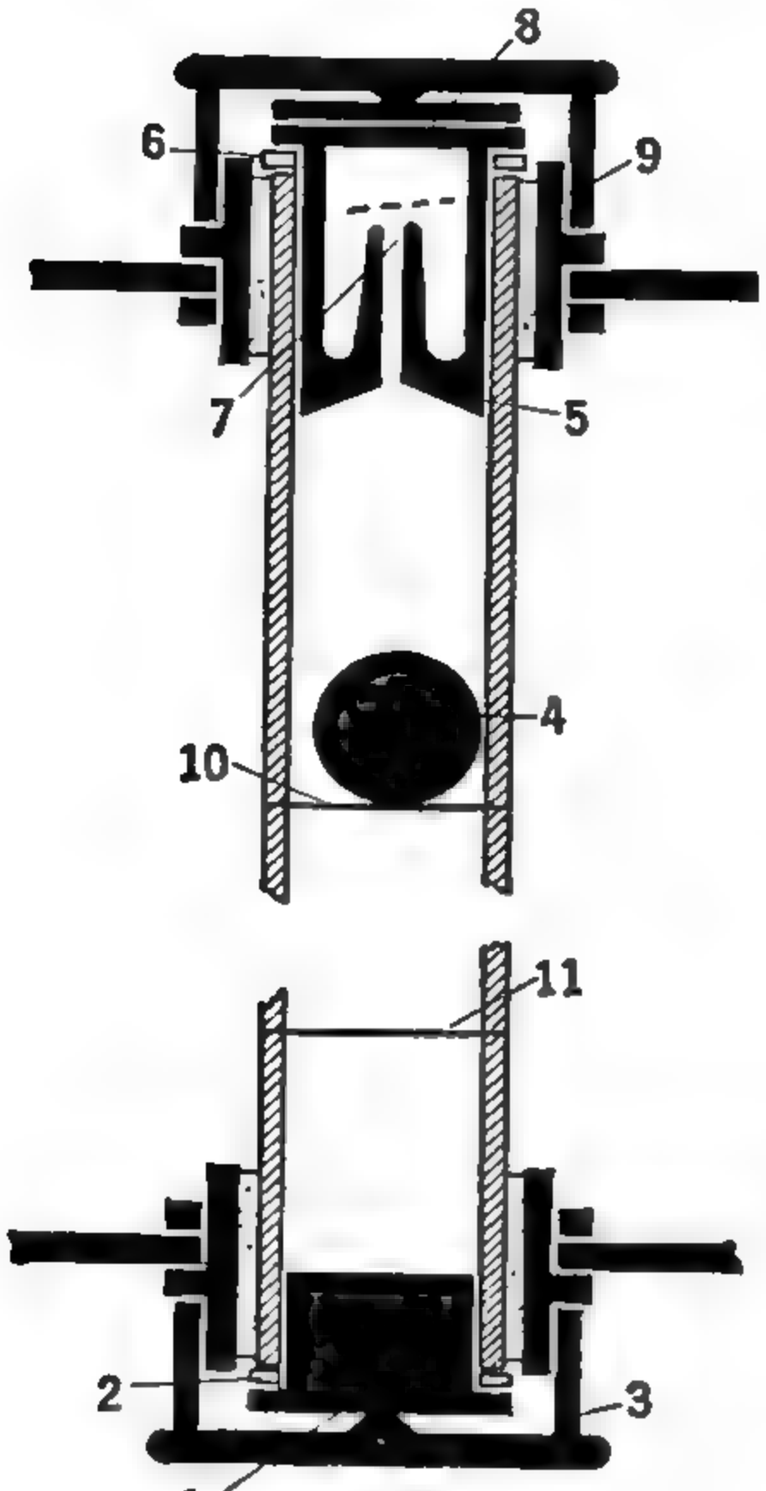


(Courtesy of Fish-Schurman Corp.)

FIG. 215. Höppler viscosimeter.

The apparatus is shown in Fig. 215, and the detailed construction of the fall tube in Fig. 216. The tube, of strictly uniform bore, is made of resistance glass. It has two marks, 10 and 11, serving as reference points for measuring the dropping time. Plugs 1 and 5, which come in contact with the liquid, are gold plated. Plug 5 has a capillary bore, to eliminate air bubbles. The fall tube is surrounded by a water bath, for temperature control; it is equipped with an electric heating unit and a pneumatic stirring device. Much closer temperature control, to $\pm 0.001°$ C., can be maintained by means of the Höppler ultrathermostat, shown in Fig. 217. The temperature of the bath is measured with a precision thermometer of the desired range, reading to 0.02° C. The entire instrument is fastened to a stand in such a way that it can be quickly turned 180°, and back again to its original position. Accurately machined balls of varying sizes are furnished with the apparatus. One is made of glass, and is used for measuring the viscosity of water and dilute solutions. The others are of

special steel, and cover a range from about 6 centipoises to 10,000 poises. For any particular liquid a ball should be chosen which gives a dropping time between 25 and 300 seconds, measured with a precision stop watch.



(Courtesy of Fish-Schurman Corp.)

FIG. 216. Fall tube of the Höppler viscosimeter.

The solutions to be measured are first freed of suspended matter by filtration or by passing them through a fine sieve. Highly viscous materials are slightly warmed and recooled, to remove occluded air. With the fall tube in the position shown in Fig. 216, the stopper 5 with the capillary is removed, and the sample, about 35 ml., is poured in so that it fills the tube to about 2 cm. from the top. The ball is introduced and any air bubbles adhering to it are removed by means of a glass rod. When stopper 5 is inserted, the level of the liquid should be about 4 mm. above the top of the capillary, to allow room for expansion. The upper end is now closed with the plate and screw cap.

After the whole apparatus has been carefully leveled, the instrument is turned 180°, and the ball drops into its initial position. After it has been made sure that the temperature is constant, the instrument is inverted again, and the level is rechecked. As soon as the lowest point of the ball passes the upper mark the time is noted, and again when it passes the lower mark.

With dark-colored materials like molasses the observations cannot be made in this manner. With such materials the passing of the equator of the ball across the mark is taken as the reference point. A special observation screen marked with a black stripe is used for this purpose. The screen is held so that when the ball approaches the mark the stripe is reflected on the surface of the ball. The screen is turned until the image of the stripe is parallel with the calibration mark, the eye being level with the mark so that it appears as a straight line. At the moment that the equator of the ball passes the mark, the mark coincides with its reflected image on the ball and with the reflected image of the stripe on the screen. This moment is registered with the stop watch, and the operation is repeated at the lower mark.

The measurements can be repeated as often as desired by inverting the instrument and turning back to the original position. For a fuller

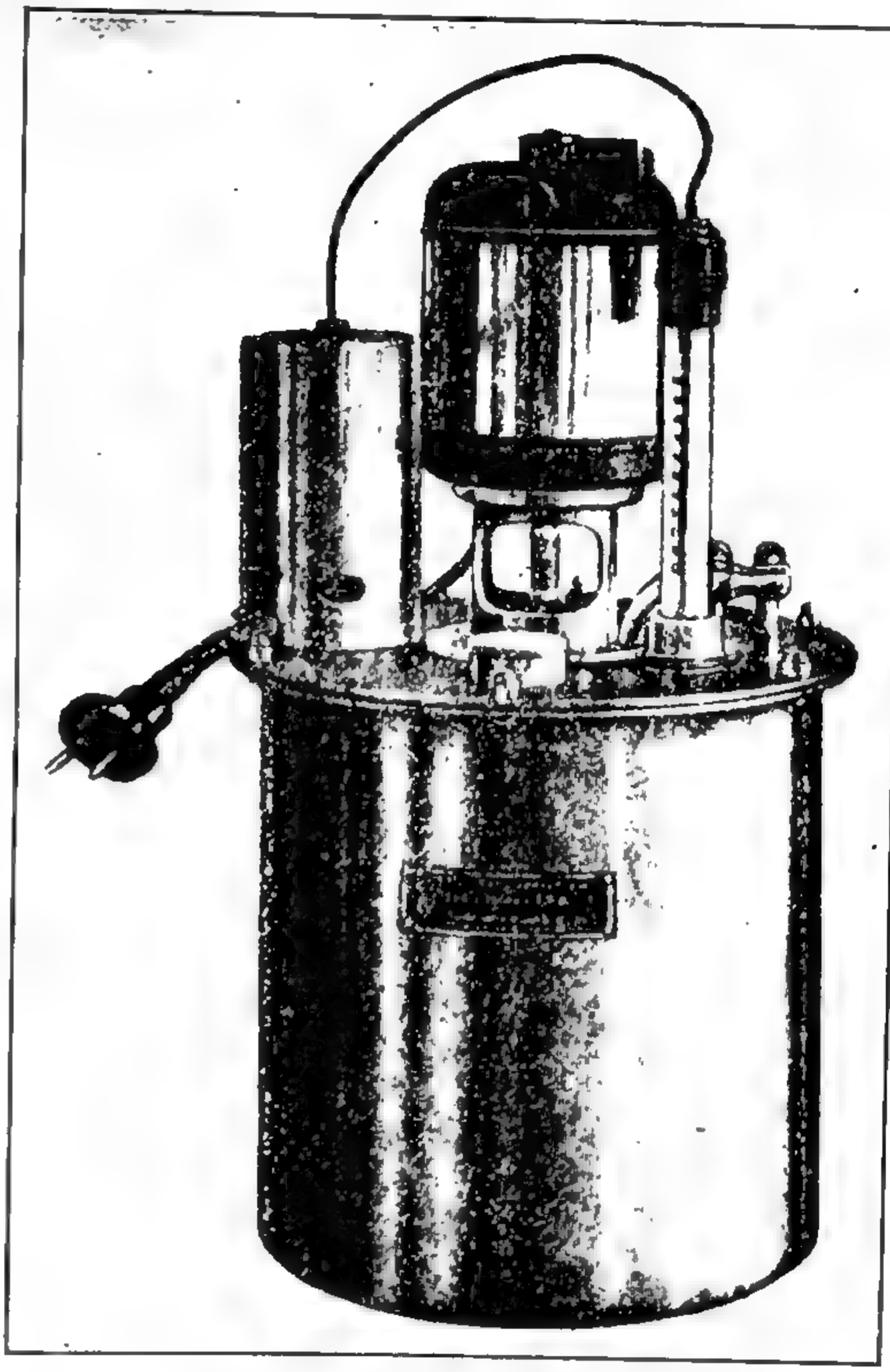
description of the mode of operation and of the care of the instrument, the directions given by the manufacturers should be consulted.

The instrument is calibrated with liquids of known viscosity, and the constant C calculated by the formula

$$C = \frac{\eta}{t\,(D - d)}$$

where t, D, and d have the same meaning as in the formula on p. 502. C being known, the dynamic viscosity of the sample is found by solving for η.

The precision of the Höppler viscosimeter has been found to range from ± 0.1 to ± 1 per cent, with an average of ± 0.27 per cent, compared to ± 2 per cent for the Vogel-Ossag instrument. It far exceeds the precision of the usual technical viscosimeters. If the kinematic viscosity in stokes is desired, it is only necessary to divide the results, in poises, by the density of the liquid.



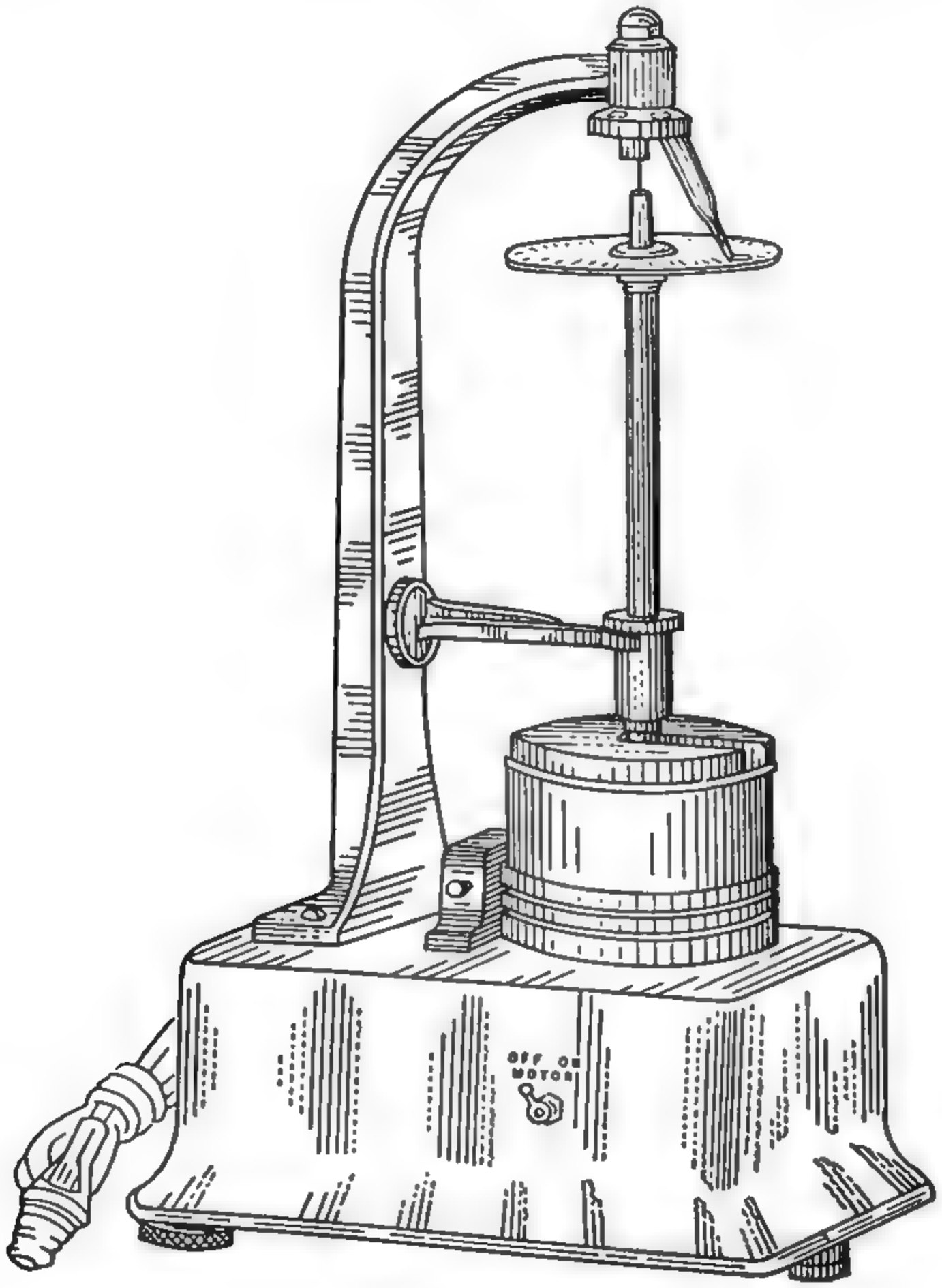
(*Courtesy of Fish-Schurman Corp.*)

FIG. 217. Höppler ultrathermostat.

A falling-ball viscosimeter especially designed for heavy final cane molasses has been described by Fabius.[7] The distance traversed by the ball is marked by two pairs of electrodes, one pair near the top and the other near the bottom of the tube. The passing of the steel ball decreases the resistance between the electrodes, and this change is measured by means of a potentiometer system with thermionic amplification and a cathode-ray tube as indicator. The air occluded in the molasses is first removed by heating to 60° C. with continuous stirring.

[7] *Reports Assoc. Hawaiian Sugar Tech.*, 15th Annual Meeting (1936), p 263.

Torsion Viscosimeters. MacMichael's Apparatus. Staněk[8] has described an instrument based on the torsion principle. The MacMichael viscosimeter, operating in a similar manner and illustrated in Fig. 218, is used considerably for technical measurements of high viscosities in the starch, flour, baking, and chocolate industries. It consists of a plunger suspended by a wire and immersed in the liquid which is contained in a cup rotated at uniform speed on a turntable.



(*Courtesy of Eimer and Amend.*)

FIG. 218. MacMichael viscosimeter.

The temperature of the liquid is kept constant by an electrically heated thermostat surrounding the cup. The viscous drag between the liquid and the plunger twists the suspension wire through a constant angle at constant speed of rotation, and the amount of twist is registered on a circular dial, connected with the plunger by a hollow spindle, and reading in arbitrary degrees (1° M. = 1/300 of circle).

[8] Cf. Šandera, *Z. Zuckerind. čechoslovak. Rep.*, 52, 1 (1927/28).

Viscosities corresponding to less than 1 to more than 2600 poises may be measured by using either a large cup with a disk plunger, or a small cup with a cylindrical plunger, by varying the speed of rotation, by varying the quantity of liquid in the cup, and by employing wires of varying gauge.

Like the falling-sphere viscosimeters, this apparatus measures dynamic viscosity. The density of the liquid does not enter into the calculation, but the ratio between degrees M. and poises varies for the different set-ups. The instrument is therefore not suitable for determinations of absolute viscosities, and it is best to standardize each individual wire with a liquid of known viscosity, as close as possible to that of the sample. The wires must be carefully handled; errors are likely to be caused by a semipermanent deformation due to imperfect elasticity.

Viscosity of Pure Sucrose Solutions. The viscosity of pure sugar solutions rises at first slowly with concentration, and then more and more rapidly. An increase in temperature causes a lowering of the viscosity at constant concentration; the decrease for each degree in temperature is considerable at low temperatures, especially when the concentration is high, but is small at high temperatures.

For dilute solutions the relationship between viscosity and concentration may, according to Arrhenius,[9] be expressed by the equation

$$\eta = A^c$$

or

$$\log_e \eta = c \times \log_e A$$

in which A is a constant and c the concentration.

But for concentrated sugar solutions the above relationship does not hold. Orth[10] made measurements at concentrations from 60° to 76° Brix and at temperatures from 20° to 90° C., and derived several formulas expressing the relationships found by him. Orth's work was carried out with instruments of low precision, and his formulas are of only historical interest. Equations, based on more recent measurements with supersaturated solutions have been given by Taimni.[11]

The most reliable figures for solutions of pure sucrose, up to 60° Brix, and from 0 to 100° C., are those of Bingham and Jackson,[12] reproduced in Table LXXIV. They were obtained with the capillary viscosimeter of Bingham.

[9] *Z. physik. Chem.*, 1, 285 (1887).
[10] *Bull. assoc. chim. sucr. dist.*, 29, 137 (1911/12).
[11] *J. Phys. Chem.*, 33, 52 (1929).
[12] *Bur. Standards Sci. Paper* 298 (1917).

TABLE LXXIV

VISCOSITIES OF PURE SUCROSE SOLUTIONS IN CENTIPOISES ACCORDING TO BINGHAM AND JACKSON

Temp., °C.	Grams Sucrose in 100 g. Solution			
	0	20	40	60
0	1.789	3.804	14.77	238
5	1.516	3.154	11.56	156
10	1.306	2.652	9.794	109.8
15	1.141	2.267	7.468	74.6
20	1.005	1.960	6.200	56.5
25	0.894	1.704	5.187	43.86
30	0.802	1.504	4.382	33.78
35	0.720	1.331	3.762	26.52
40	0.653	1.193	3.249	21.28
45	0.596	1.070	2.847	17.18
50	0.550	0.970	2.497	14.01
55	0.507	0.884	2.219	11.67
60	0.470	0.808	1.982	9.83
65	0.436	0.742	1.778	8.34
70	0.406	0.685	1.608	7.15
75	0.379	0.635	1.462	6.20
80	0.356	0.590	1.334	5.40
85	0.334	0.550	1.221	4.73
90	0.315	.....	1.123	4.15
95	0.298	.....	1.037	3.72
100	0.282	.....	0.960	3.34

The viscosities of sucrose solutions above 60° Brix have been determined by various investigators, notably by Bennett and Nees[13] with their falling-sphere instrument, and by Landt[14] with the Höppler viscosimeter. Landt's results are shown in Table LXXV.

An explanation of the large effect of concentration and temperature on the viscosity of sucrose solutions has been offered by Spengler and Landt[15] on the basis of conductance measurements on the one hand, and the solvation theory of Fikentscher and Mark on the other. It is postulated that the sucrose molecules form hydrated aggregates at high concentration and low temperature, and that these aggregates cause higher viscosity since the available quantity of uncombined water is greatly diminished.

Coumou[16] has observed that the viscosity of freshly prepared and cooled supersaturated solutions of sucrose decreases upon standing, but becomes constant after about 2 hours. Rapid stirring increases

[13] *Ind. Eng. Chem.*, **22**, 91 (1930).
[14] *Centr. Zuckerind.*, **44**, 102 (1936).
[15] *Z. Ver. deut. Zucker-Ind.*, **82**, 545 (1932).
[16] *Chem. Weekblad*, **33**, 542 (1936).

the viscosity of such solutions, but the viscosity returns to normal upon standing. This abnormal behavior must be considered in viscosity determinations on sucrose solutions, and it has an important bearing also on manufacturing operations where highly concentrated sugar solutions are agitated or conveyed by pumping.

TABLE LXXV

VISCOSITIES OF PURE SUCROSE SOLUTIONS IN CENTIPOISES, ACCORDING TO LANDT

Sucrose, Per Cent by Weight	Degrees Centigrade						
	20	30	40	50	60	70	80
60	57.2	33.1	20.6	13.7	9.5	6.9	5.3
61	67.9	38.6	23.8	15.6	10.7	7.7	5.8
62	80.9	45.4	27.5	17.8	12.1	8.6	6.4
63	97.0	53.6	32.0	20.4	13.7	9.6	7.1
64	117	63.6	37.4	23.5	15.6	10.8	7.9
65	143	76.0	44.1	27.2	17.9	12.2	8.8
66	176	91.6	52.2	31.7	20.6	13.9	9.9
67	219	111	62.2	37.2	23.9	15.9	11.2
68	276	137	74.7	44.0	27.9	18.3	12.7
69	354	170	90.6	52.4	32.8	21.2	14.5
70	460	214	111	63.1	38.8	24.8	16.7
71	614	274	138	76.8	46.3	29.2	19.4
72	.....	356	174	94.5	55.9	34.8	22.7
73	.....	470	222	118	68.2	41.8	26.8
74	.....	631	289	149	84.1	50.7	31.9
75	.....	864	381	190	105	62.0	38.4
76	.....	1214	513	246	133	76.8	46.6
77	.....	.....	701	323	171	96.1	57.2
78	.....	.....	980	433	222	122	71.1
79	.....	.....	1430	593	293	156	89.6
80	.....	.....	2160	832	394	204	115
81	.....	.....	.....	1200	546	272	151
82	.....	.....	.....	1800	770	373	200
83	.....	.....	.....	.....	1125	519	270
84	.....	.....	.....	.....	1700	740	376

Viscosity of Impure Sucrose Solutions. This subject, which is of the greatest importance in practical sugar-house work, has been studied by a number of investigators, particularly by Claassen, Orth, Bennett and Nees, Spengler, and Landt.[17] Generally speaking, at ordinary temperatures sugar solutions containing inorganic and organic salts are less viscous than pure sucrose solutions of the same concentration. The differences are the greater the lower the purity and the higher the concentration. But as the temperature increases the differences become smaller and smaller, and at high temperature and high concentration the viscosity of an impure sugar solution may even exceed that

[17] *Centr. Zuckerind.*, 44, 102 (1936).

of a corresponding pure solution. The effect on the viscosity varies with the nature and quantity of the non-sugars present. Organic non-sugars, among them raffinose, usually produce higher viscosity than inorganic non-sugars, but invert sugar lowers the viscosity. Among inorganic impurities, neutral salts increase the viscosity less than added sucrose; potassium salts have the least effect, sodium salts somewhat more, while calcium salts tend to increase the viscosity markedly. The reaction of the medium does not influence the viscosity to any extent between *p*H 2 and 11, but above *p*H 11 the viscosity rises rapidly.

Viscosities of technical beet-house sirups, varying from 60 to 80 purity, from 70° to 86° Brix, at 40° to 70° C., have been reported by Grut.[18] It was found that even at the same Brix, the same temperature, and practically the same purity, the viscosity of beet molasses produced by different factories in the same year may vary as much as 37 per cent from the average figure.

The high viscosity caused by gums, such as dextran or levan, sometimes occurring in sugar products, has a detrimental effect in the work of evaporating and boiling to grain. Since the viscosity increases rapidly with supersaturation, as may be seen from Table LXXV, the successful sugar boiler aims to prevent excessive supersaturation. From the viscosity standpoint alone, it is best to boil at as high a temperature as practicable.

The determination of viscosity is of great value in certain branches of analytical work, as, for example, the examination of commercial dextrins, for which see p. 1133.

Plasticity. Heavy, semisolid pastes, made by boiling starch with water, begin to flow through capillaries only after the pressure applied exceeds a certain minimum value. This type of flow is termed plastic flow, in contradistinction to viscous flow. The colloid-chemical changes taking place in the preparation of starch paste are rather complicated, there being at least four different stages in the process. When the starch is mixed with cold water an ordinary suspension forms which upon heating gelatinizes and thickens. During the boiling period the starch cells are ruptured, with consequent thinning of the paste. Upon cooling the paste thickens again. For methods for measuring the consistency of starch pastes the chemist is referred to the work of Porst and Moskowitz,[19] of Ripperton,[20] and of Caesar.[21]

[18] *Z. Zuckerind., čechoslovak. Rep.*, **61**, 445 (1936/37).
[19] *Ind. Eng. Chem.*, **14**, 49 (1922).
[20] *Ind. Eng. Chem., Anal. Ed.*, **3**, 152 (1931).
[21] *Ind. Eng. Chem.*, **24**, 1432 (1932); **27**, 1447 (1935).

SPECIFIC HEAT OF COMBUSTION

Units Employed in Calorimetry. The number of calories or heat units which a substance gives off, when burned in oxygen under specified conditions, is a constant which has been extensively used in the investigation of sugars. The determination has been especially employed in studying the calorific value of the different carbohydrates which are used in foods.

The Small, or Gram, Calorie (cal.) is defined as the quantity of heat necessary to raise 1 g. of water through 1° C. The quantity of heat necessary to raise 1 g. of water from 0° to 1° C. is not, however, exactly the same as that necessary to raise 1 g. of water from 99° to 100° C. It is therefore necessary to specify the temperature at which the measurement has been made. The temperatures generally used are 15°, 18°, or 20° C., and the corresponding calories are defined by subscripts, e.g., $cal._{15}$, $cal._{18}$, $cal._{20}$. The so-called mean calorie is one one-hundredth of the heat required to raise 1 g. of water from 0° to 100° C.

The Large, or Kilogram, Calorie (Cal.) equals 1000 small calories, and may be defined, with the limitations previously noted, as the quantity of heat necessary to raise 1000 g. of water through 1° C.

In order to place the results of heat measurements on a uniform basis, and permit comparisons of determinations made at different temperatures, many investigators prefer to express heat values in equivalent units of work performed. In this system 1 $cal._{15}$ equals 4.185 joules, 1 $cal._{20}$ = 4.181 joules, and the mean calorie = 4.186 joules.

The Bomb Calorimeter

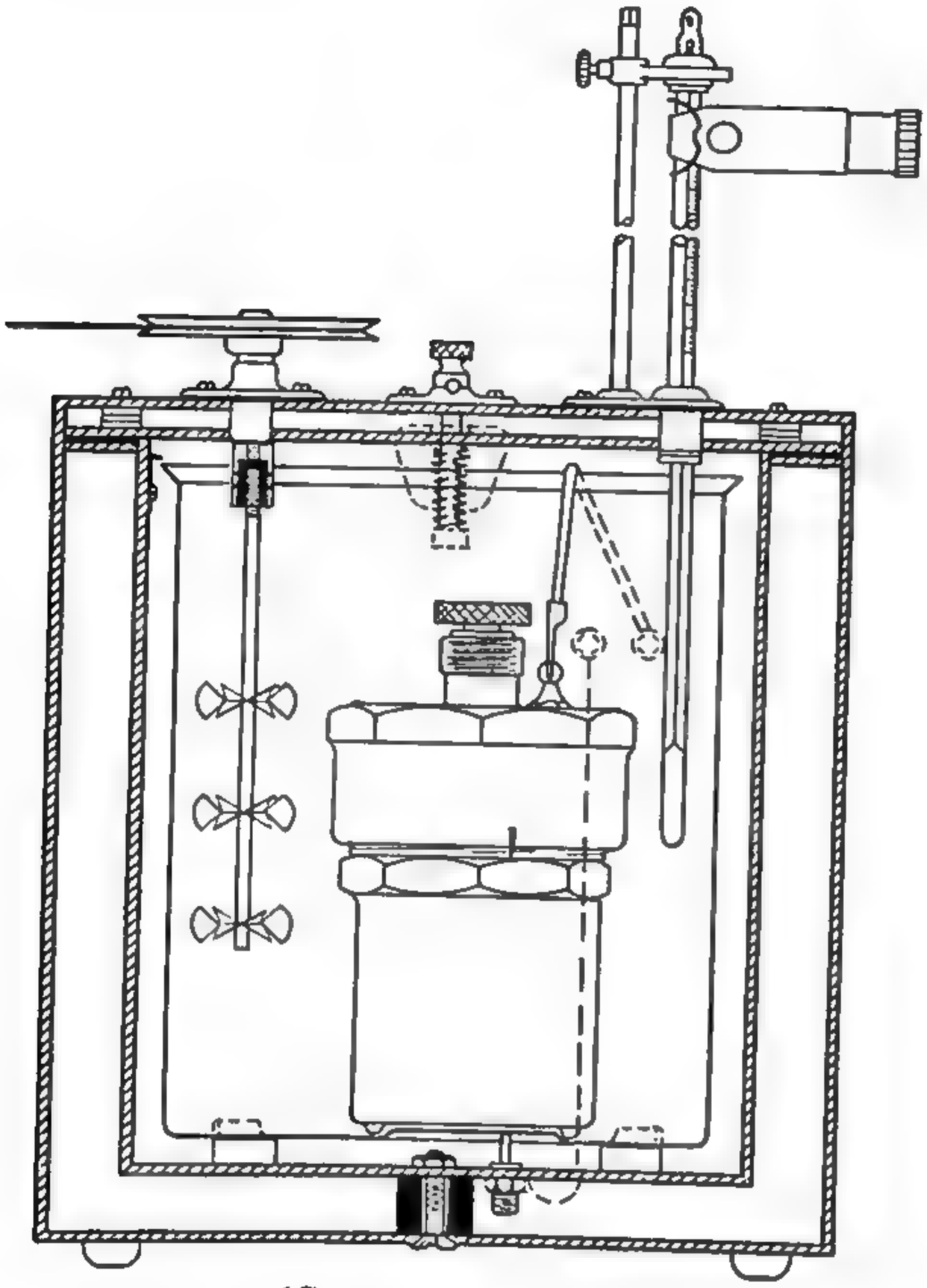
The determination of calories of combustion is usually made by means of a bomb calorimeter, first employed for this purpose by Andrews.[22] Later Berthelot[23] re-invented the apparatus, introduced the use of compressed oxygen, and extensively applied his method to a variety of heat measurements. The original bomb of Berthelot, on account of the large amount of platinum it contains, is exceedingly expensive, and has been variously modified by Mahler, Hempel, Atwater, Parr, and others for the purpose of reducing the cost.

Two different procedures are commonly followed to determine heats of combustion. In the first or "ordinary" method, the temperature

[22] *Pogg. Ann.*, 75, 27 (1848).

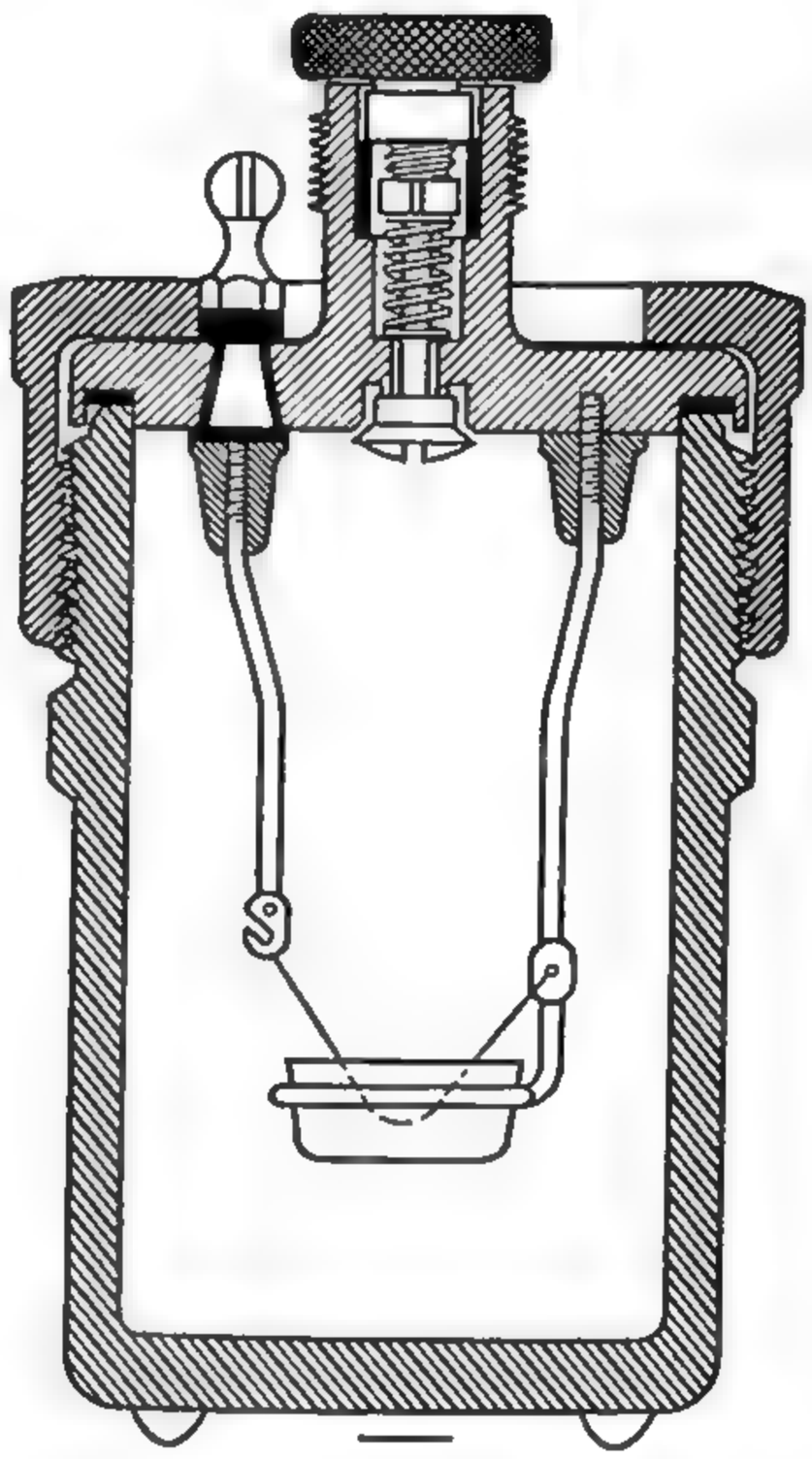
[23] "Traité pratique de calorimétrie chimique"; also *Ann. chim. phys.*, [6] 6, 546 (1885).

of the jacket surrounding the calorimeter is kept constant, and the rise of temperature in the calorimeter is observed at regular intervals. In the "adiabatic" method, developed principally by Richards and his coworkers,[24] the temperature of the jacket is kept equal to that of the calorimeter and is measured only at the beginning and the end of the experiment.



(Courtesy of Parr Instrument Co.)

FIG. 219. Showing construction of Burgess-Parr oxygen bomb calorimeter.



(Courtesy of Parr Instrument Co.)

FIG. 220. Showing details of the bomb for the Burgess-Parr oxygen bomb calorimeter.

Ordinary Method. Parr Oxygen Bomb Calorimeter. This apparatus, extensively used for the determination of heats of combustion, is shown in Fig. 219. The most important feature of the calorimeter is the bomb (Fig. 220), made in this case of a special alloy, illium, which is resistive to attack by the products of the combustion. Its principal constituents are nickel, copper, tungsten, and chromium. The cover is provided with a gasket of an asbestos compound, imbedded in a groove and resting on the rim of the cup, and is held in

[24] *J. Am. Chem. Soc.*, 31, 1275 (1909), and subsequent papers.

place by a forged-steel collar which is screwed tightly into position by means of a spanner wrench. The valve at the top of the bomb cover serves to admit the oxygen from a tank and shuts automatically when the pressure is released. The cover is also, on its inner surface, provided with two stiff terminal rods, one of which ends in a ring support for the combustion capsule, made of illium. The iron wire for igniting the charge is suspended between the terminal rods. About a gram of the material to be burned is placed in the capsule and the ignition wire located to touch the top of the charge. With sugar and similar carbohydrates which do not ignite readily the wire should be bent into a spiral to make contact with the charge at several points. It is still better to press the substance into a pellet with a special pellet machine and imbed the iron wire in the pellet. One of the terminal rods is in metallic contact with the bomb, and a connection at the bottom of the bomb leads to a binding post on the outer jacket. The other rod is insulated from the bomb and has a direct electrical connection with the second binding post outside.

Operation of the Calorimeter. After the charge is introduced, the bomb is filled with pure oxygen under 25 to 30 atmospheres pressure and then placed in the calorimeter vessel which rests in the double-walled Bakelite jacket, insulating the bomb system from the outside atmosphere. The wires for igniting the charge are connected, but the switch left open. Then 2000 g. of water at a temperature about 1° to 2° lower than that of the room is added, and the cover placed on the calorimeter. The thermometer furnished with the Parr calorimeter is graduated in twentieths of a degree Fahrenheit and is read with a lens to estimate subdivisions. The readings may be converted into Centigrade to express the heat of combustion in calories. But for this type of work a Beckmann thermometer graduated to 0.01° C., and read to 0.001° C. by means of a lens, is to be preferred; it should be provided with an official certificate giving the necessary scale corrections. The mercury thread of the thermometer is adjusted at the desired point by partly filling or emptying the reservoir.

After the cover has been placed on the calorimeter, the mechanical stirrer is set in motion and adjusted to about 150 revolutions per minute. The thermometer is now read at intervals of 1 minute, the top being tapped gently with an electric hammer or lead pencil before each reading to prevent lagging of the mercury thread. When five successive readings show a uniform rise in temperature, the electric switch is closed exactly at the end of the fifth minute, and a current of 2 to 4 amperes at 10 to 12 volts is passed through the ignition wire. It is advisable to place a small electric lamp in the resistance circuit,

and as soon as this is extinguished, indicating the fusion of the wire, the switch is reopened to avoid heating the water by the current. The thermometer readings are continued and noted at the end of each minute until the maximum elevation of mercury is reached and the rate of fall has become regular. Equilibrium is usually obtained within 5 or 6 minutes. After that readings are taken for a final period of 5 minutes, when the calculation may be made.

Standardization of the Calorimeter. Hydrothermal Value. The calories of combustion are calculated from the observations of a calorimeter experiment by multiplying the hydrothermal value (in grams) of the calorimeter system by the corrected rise in temperature and dividing the product (after subtracting the heat units due to accessory combustions) by the weight in grams of the substance taken.

TABLE LXXVI

COMPUTED HYDROTHERMAL VALUE FOR PARR OXYGEN BOMB CALORIMETER

Material	Weight	Specific Heat	Water Equivalent
	grams		grams
Illium	2510.0	0.1120	281.0
Stainless steel	655.0	0.1190	77.9
Brass	881.0	0.0883	77.8
Nickel	41.0	0.1030	4.2
Rubber	12.0	0.3310	3.9
Oxygen (30 atm.)	14.9	0.1570	2.3
Water	2001.0	0.9975	2000.0
Total	.....	.....	2447.1

The accuracy of all calorimetric experiments is dependent upon the exactness with which the hydrothermal value of the calorimeter is known. Several methods for computing the water equivalent of the calorimeter system may be employed. The method generally followed in practice is calibration by means of a standard substance whose heat of combustion is accurately known. The internationally accepted primary standard is benzoic acid, whose heat of combustion is 6324 $cal._{15}$ per gram weighed in air. As a secondary standard salicylic acid, with 5242 $cal._{15}$ per gram weight in air, has been suggested.

In the "additive" calibration method of a calorimeter system the weight of each part is multiplied by its specific heat, and the sum of these products is taken as the hydrothermal value of the entire system. An example of such a calculation, furnished by the Parr Instrument Company for an oxygen bomb calorimeter selected at random, is shown in Table LXXVI.

For a detailed discussion of methods for determining hydrothermal values reference should be made to the literature quoted by Kharasch.[25]

Correction for Radiation. When the conditions of the experiment are properly controlled the calorimeter system at the beginning of combustion is slightly cooler, and at the end of combustion slightly warmer, than the surrounding air. During the first period the calorimeter gains heat, and in the second loses heat to the surrounding air; the thermometer readings must be corrected, therefore, for the errors of radiation. This correction C is made by the Regnault-Pfaundler[26] formula

$$C = nV + \frac{V' - V}{\theta' - \theta}\left(\frac{\theta_n + \theta_0}{2} + \sum_1^{n-1} \theta - n\theta\right)$$

where n = number of time units (minutes) in combustion period.
V = rate of fall of temperature of calorimeter during initial period. (The change is actually a rise but for convenience is expressed as a fall, the value of V thus being negative.)

V' = rate of fall of temperature of calorimeter during final period.
θ = mean temperature of calorimeter during initial period.
θ' = mean temperature of calorimeter during final period.

$\theta_1, \theta_2, \ldots \theta_n$ = temperature at end of first, second, . . . nth minutes of combustion period.

θ_0 = temperature at moment of ignition.

Illustration of Method. The application of the formula is best understood from a special case and the following example of the combustion of sucrose is taken from a paper by Atwater and Snell.[27] The calorimeter employed had a water equivalent of 2100 g. The data of the experiment are given in the following record, which is a convenient form for determinations of this kind.

[25] *Bur. Standards J. Research*, 2, 361 (1929).
[26] Pfaundler, *Pogg. Ann.*, 129, 113 (1866).
[27] *J. Am. Chem. Soc.*, 25, 659 (1903).

Sample No.	Description Cane Sugar.	Date, July 13, 1901.
Bomb No. 3	Observer, J. F. Snell.	Thermometer, No. 733

Capsule No. 1
Wt. caps. + subs. = 4.2501
Wt. capsule = 2.8783
Wt. substance, W = 1.3718

Correction for Accessory Combustions.
Wt. Fe 13.0 − 1.1 = 11.9 mg. = 19.0 cal.
Wt. naphthalene = 6.4 mg. = 61.6 cal.
HNO_3 = 6.6 cal.
Correction for accessories = 87.2 cal.

		Readings	*Corrected readings*
Initial period	1	1.018	1.015
	2	1.021	
	3	1.025	
	4	1.027	
	5	1.030	
	6 θ_0	1.032	1.029
Main period	7 θ_1	2.300	2.3
	8 θ_2	3.650	3.7
	9 θ_3	3.678	3.7
	10 θ_4	3.662	3.7
	11 θ_5	3.653	
			13.4
	$\frac{\theta_5 + \theta_0}{2}$	= 2.3	
Final period	Sum	= 15.7	
	5 θ	= 5.1	
	Diff.	= 10.6	
	Log diff.	= 0253	
	Log $V' - V$	= 7324	
	Colog. $\theta' - \theta$	= 5820	
			3397
	Antilog.	= +0.0219	
	+5 V	= −0.014	
	Radiation correction	= +0.0079	
	16 3.640		3.633
	Time 3.30		

Initial period

Fall = −0.014
Rate V = −0.0028
Mean $t°, \theta$ = 1.022

Corrected reading

θ_5 = 3.646
θ_0 = 1.029
$\theta_5 + \theta_0$ = 4.675
$\frac{1}{2}$ = 2.3

Final period

Fall = + .013
Rate V' = + .0026
V = − .0028
$V' - V$ = + .0054

Mean $t°, \theta'$ = 3.640
θ = 1.022
$\theta' - \theta$ = 2.618

Thermometer correction

$T°$ air = 25.2
$T°$ water = 23.8
1st reading = 1.0
$T°$ of zero = 22.8
Corr. for 1° = + .001
Rise (degrees) = 2.6
Ther. corr. = + .0026

Final calculations

θ_5 = 3.646
θ_0 = 1.029
$\theta_5 - \theta_0$ = 2.617
Th. corr. = + .0026
Rad. corr. = + .0079
Corr. rise = 2.6275
Corr. rise × 2100 = total heat = 5517.8
Accessories = 87.2
Corrected heat = 5430.6
Log corr. heat = 73485
Log W = 13729
59756
Heat of combustion per gram = 3959

Applying the formula to the above example, where the number of time units, n, is 5, we obtain, for the several expressions, $V = -0.0028$ and $nV = -0.014$; $\frac{V' - V}{\theta' - \theta} = \frac{+.0054}{2.618}$; $\frac{\theta_n + \theta_0}{2} = 2.3$; $\sum_1^{n-1} \theta = 13.4$; and $n\theta = 5.1$. The combination of these values in the formula gives a radiation correction of $C = +0.0079°$.

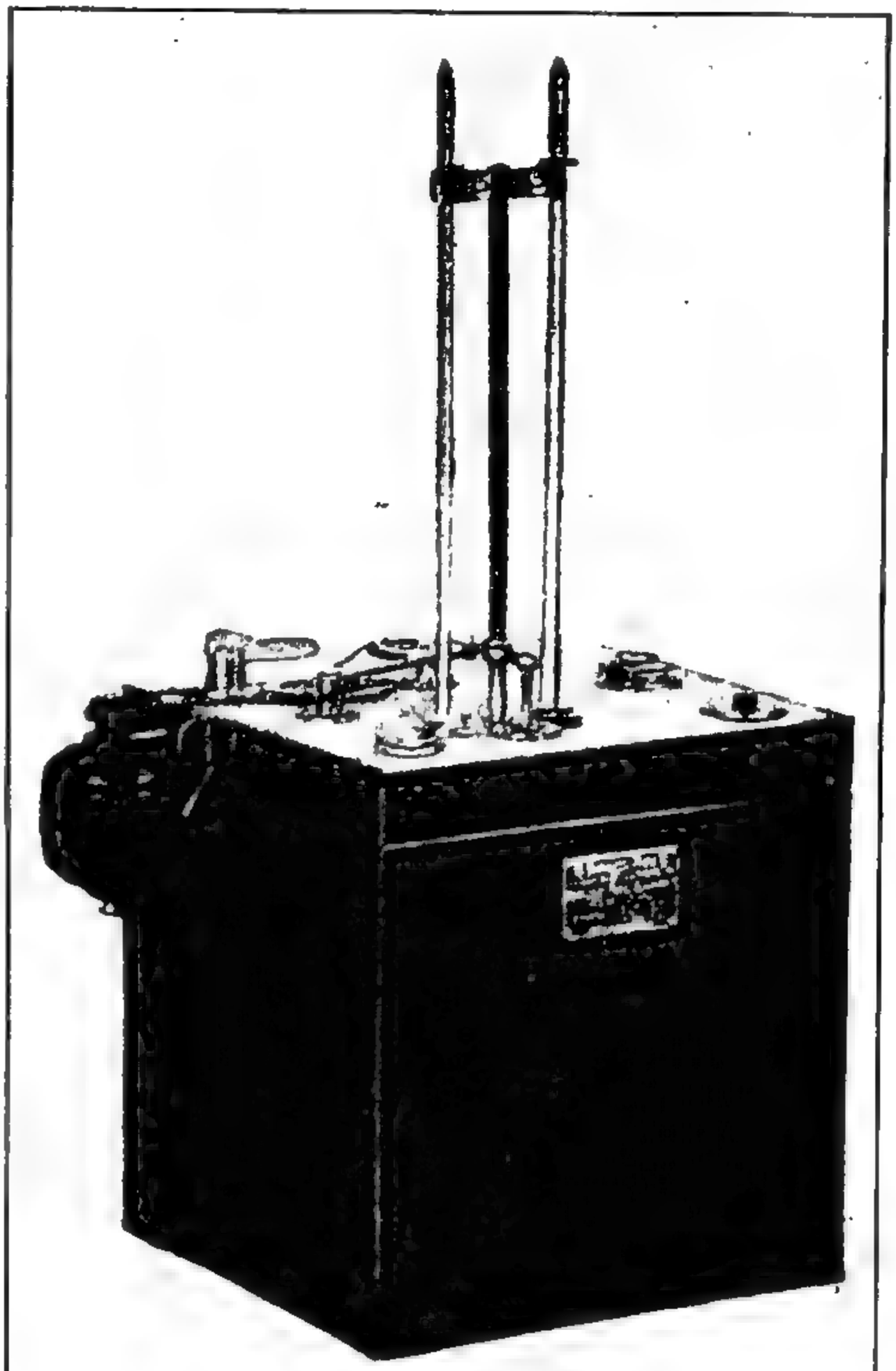
The corrected rise of the Beckmann scale was 2.617°, and this corrected to true degrees Centigrade and for radiation gives 2.6275° C. as the corrected rise in temperature, which, multiplied by 2100, the water equivalent of the calorimeter, gives 5517.8 calories.

Correction for Accessory Combustions. The weight of the iron wire was 13 mg. The quantity unburned was 1.1 mg. The quantity burned was

therefore 11.9 mg. The specific heat of combustion of iron being 1601 cal., the heat of combustion of 11.9 mg. is $11.9 \times 1.6 = 19$ cal. The quantity of naphthalene burned was 6.4 mg., which yields $6.4 \times 9.63 = 61.6$ cal., the specific heat of combustion of naphthalene being 9628 cal. The heat of combustion of nitrogen in the bomb as determined by titration of the nitric acid is 6.6 cal. ($N_2 + O_5 + H_2O = 2\ HNO_3$; 0.004406 g. HNO_3 = 1 cal.). The total heat from accessory combustions is, therefore, $19 + 61.6 + 6.6 = 87.2$ cal.

Deducting this quantity from the total heat set free in the apparatus, we have $5517.8 - 87.2 = 5430.6$ cal. as the heat due to the combustion of the sugar. The quantity of sugar burned was 1.3718 g. The specific heat of combustion according to this determination is, therefore, $5430.6 \div 1.3718 = 3959$ cal.

Simplified modifications of the Regnault-Pfaundler formula have been proposed by White,[28] and the American Society for Testing Materials has adopted a still simpler method to correct for the radiation losses.[29]



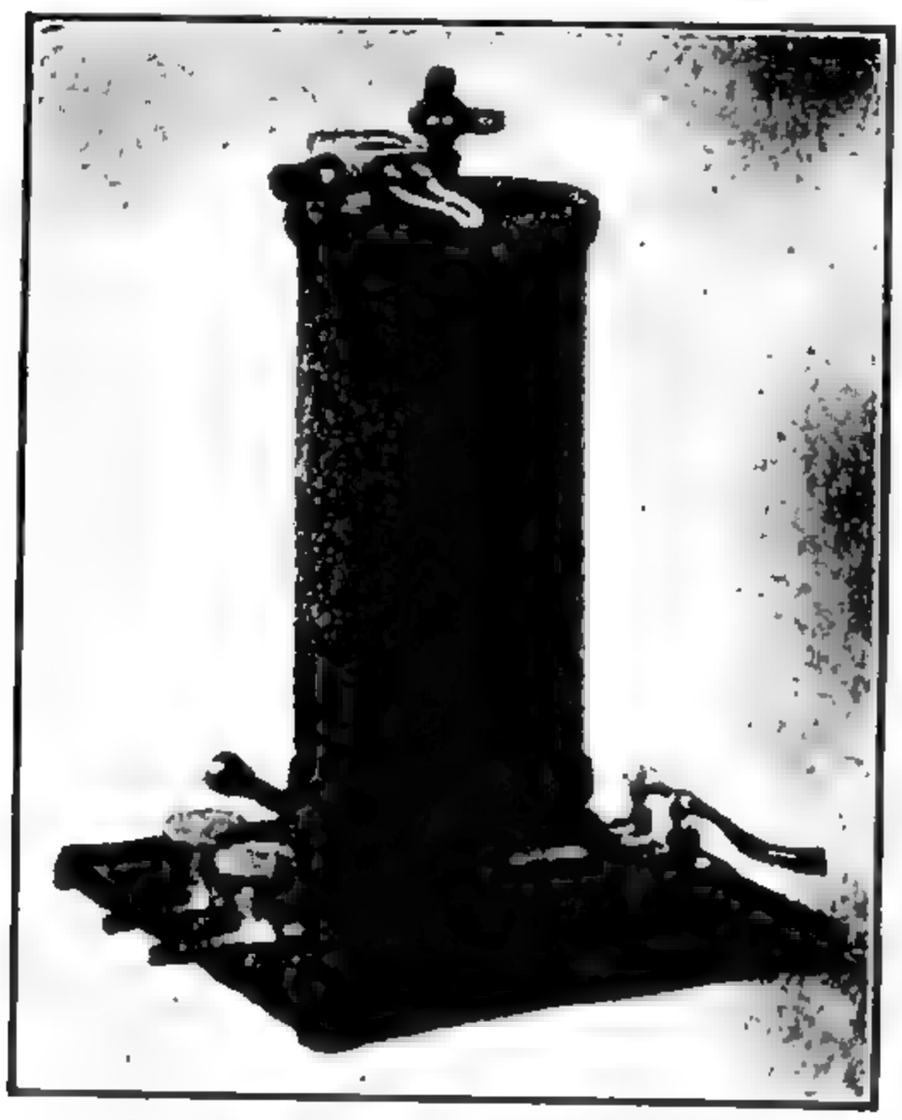
(*Courtesy of Parr Instrument Co.*)

FIG. 221. Burgess-Parr adiabatic calorimeter.

Parr Adiabatic Calorimeter. Corrections for radiation losses are avoided in this type of instrument, the losses being counterbalanced by equalizing the temperature in the calorimeter vessel and the outer jacket. This is accomplished in the Parr adiabatic calorimeter, Fig. 221, by circulating water of the required temperature through the outer jacket which is separated from the calorimeter vessel by an air space, and is provided with a stirrer. The temperature of the water in the

[28] *J. Am. Chem. Soc.*, 48, 1146 (1926).
[29] "A. S. T. M. Book of Standards," 1929.

jacket is measured with a second thermometer of the same type as that used in the calorimeter vessel, and is regulated by means of a thermostatically controlled electric water heater, Fig. 222; a direct cold-water connection is also provided.



(*Courtesy of Parr Instrument Co.*)

FIG. 222. Water heater for Burgess-Parr adiabatic calorimeter.

The method of assembling the apparatus is similar to that in the ordinary method. The outer jacket is filled with cold water, and its temperature is so regulated, by admitting hot or cold water as required, that the two thermometers show the same reading. When the readings become identical for 2 or 3 minutes, the temperature recorded is taken as the initial temperature. The charge is fired and the temperature rise in the calorimeter vessel is paralleled as closely as possible by regulating the temperature of the water in the jacket. When equilibrium is again established for 2 to 3 minutes, the temperature is recorded as the final reading. The temperature rise due to the combustion can then be found directly by subtracting the initial reading, corrected only for the thermometer error, from the final reading, corrected in the same manner. From the temperature rise, which requires no correction for radiation losses, the heat of combustion is computed exactly as in the ordinary method.

Gram-Molecular Heat of Combustion. The gram-molecular heat of combustion is found by multiplying the calories per gram by the molecular weight (M). To avoid large figures it is customary to express this unit in terms of large calories.

$$\text{Gram-mol. Cal.} = \frac{\text{cal.} \times M}{1000}$$

CALORIFIC CONSTANTS OF DIFFERENT SUGARS

In Table LXXVII, taken from the compilation by Kharasch,[30] the calorific constants, reduced to weights in vacuo, are given for the principal sugars, polysaccharides, and sugar alcohols. Where several values are given, they were obtained by different investigators.

In later determinations Huffman and Fox[31] obtained 3720.0 cal. for

[30] *Bur. Standards J. Research*, **2**, 361 (1929).

[31] *J. Am. Chem. Soc.*, **60**, 1400 (1938).

TABLE LXXVII

HEATS OF COMBUSTION OF SUGARS, POLYSACCHARIDES, AND SUGAR ALCOHOLS

	Small Calories, 1 g.	Large Calories for 1 Gram Molecule
Sugars		
Arabinose, $C_5H_{10}O_5$	{3718.0 3730.7}	{558.0 559.9}
Xylose, $C_5H_{10}O_5$	{3741.4 3734.7}	{561.5 560.5}
Rhamnose, $C_6H_{12}O_5$	{4374.7 4377.2}	{717.9 718.3}
Rhamnose, (crystd.), $C_6H_{12}O_5 \cdot H_2O$	3907.0	711.5
Fucose, $C_6H_{12}O_5$	4338.2	711.9
Glucose, $C_6H_{12}O_6$	3736.9	673.0
Galactose, $C_6H_{12}O_6$	{3717.4 3724.1}	{669.5 670.7}
Fructose, $C_6H_{12}O_6$	{3751.3 3725.7}	{675.6 671.0}
Sorbose, $C_6H_{12}O_6$	3714.2	668.3
Sucrose, $C_{12}H_{22}O_{11}$	3944.1	1349.6
Lactose, $C_{12}H_{22}O_{11}$	3947.6	1350.8
Lactose, $C_{12}H_{22}O_{11} \cdot H_2O$	3733.3	1344.7
Maltose, $C_{12}H_{22}O_{11}$	{3945.9 3949.2}	{1350.2 1351.3}
Maltose, $C_{12}H_{22}O_{11} \cdot H_2O$	3718.0	1339.2
Trehalose, $C_{12}H_{22}O_{11}$	3943.5	1349.4
Trehalose (crystd.), $C_{12}H_{22}O_{11} \cdot 2H_2O$	3547.0	1341.5
Raffinose, $C_{18}H_{32}O_{16}$	4016.9	2025.5
Raffinose, (crystd.), $C_{18}H_{32}O_{16} \cdot 5H_2O$	3396.8	2018.9
Melezitose, $C_{18}H_{32}O_{16}$	3909.8	2042.0
Polysaccharides		
Cellulose, $(C_6H_{10}O_5)_n$	4180.8	.....
Starch, $(C_6H_{10}O_5)_n$	4178.8	.....
Dextrin, $(C_6H_{10}O_5)_n$	4107.9	.....
Inulin, $(C_6H_{10}O_5)_n$	{4129.9 4190.0}	....
Glycogen, $(C_6H_{10}O_5)_n$	4186.8	.....
Sugar alcohols		
Erythritol, $C_4H_{10}O_4$	4129.3	504.1
Arabitol, $C_5H_{12}O_5$	4022.4	611.8
Mannitol, $C_6H_{14}O_6$	3995.4	727.6
Dulcitol, $C_6H_{14}O_6$	{4003.7 3974.0}	{729.1 723.7}
Perseitol, $C_7H_{16}O_7$	3940.0	835.8
Quercitol, $C_6H_{12}O_5$	4291.3	704.2
Inositol, $C_6H_{12}O_6$	3676.3	662.1

α-*d*-glucose, and 3367.7 cal. for its hydrate. Clarke and Stegeman[32] have reported the following values: β-*d*-fructose, 3732.3 cal.; α-*d*-galactose, 3704.8 cal.; *l*-sorbose, 3724.4 cal.; β-lactose, 3932.7 cal.; α-lactose hydrate, 3761.6 cal.; β-maltose hydrate, 3777.8 cal.

[32] *J. Am. Chem. Soc.*, 61, 1726 (1939).

It is seen from Table LXXVII that the molecular heat of combustion is always higher for the anhydride than for the hydrate of the same sugar. The molecular heat of combustion of the higher saccharides is also greater than the sum of the values of their components. Thus:

Sucrose		= 1349.6 g.-mol. Cal.
Glucose = 673.0 +	}	= 1346.3 g.-mol. Cal.
Fructose = 673.3 average		
Difference	=	3.3 g.-mol. Cal.

This difference may be taken as the equivalent of heat which is liberated during inversion. In the same way

Raffinose		= 2025.5 g.-mol. Cal.
Glucose = 673.0		
Fructose = 673.3	}	= 2016.4 g.-mol. Cal.
Galactose = 670.1		
Difference	=	9.1 g.-mol. Cal.

The hydrolysis of sugars, therefore, may be regarded as an exothermic reaction.

Calculation of Calories from Chemical Formulas. Various methods have been proposed for calculating the molecular heat of combustion from the chemical formula of sugars.

The older methods were based on the heat of combustion of carbon and of hydrogen. The oxygen atoms in the sugar were assumed to combine either with the carbon, or with the hydrogen, or with both proportionately, and the total heat of combustion of the uncombined atoms was supposed to represent the heat of combustion of the sugar. The results of these systems of calculation differed from the observed values by several times the experimental error and gave only rough approximations. Swietoslawski went a step further and established combustion constants for various atomic linkages, such as C—C, C—H, and C—O, by solving simultaneous equations for series of homologues, but his system also lacked a rational theoretical basis and made further assumptions necessary.

Later, Kharasch and Sher[33] succeeded in developing a complete theory, based on modern conceptions of the atom and of valence, and their heats of combustion check remarkably well with the values found by experiment. To explain this theory, the following considerations are quoted from the work of these authors. They make these postulates:

1. Heat of combustion is due to the liberation of energy in the interdisplacement or shift of electrons between atoms or molecules.

[33] *J. Phys. Chem.*, 29, 625 (1925).

2. The heat of combustion of a compound is a function of the total number of electrons interdisplaced, and must be an integral multiple of the heat value given by one electron.

3. The net amount of energy in the form of heat liberated by the interdisplacement of an electron from an arrangement such as exists in a methane type of molecule to that of the carbon dioxide type is approximately 26.05 Cal. per mole per electron. This figure is derived by dividing the gram-molecular heat of combustion of octane (1302.9 Cal.) by 50, its number of electrons.

4. The displacement of the electron from the methane arrangement to that of the carbon dioxide type occurs in stages, according to Bohr's conception of the atom. Presumably, the displacement of electrons from the valence orbit into outside orbits requires energy, while the reverse of this process liberates energy. The energy evolved in the interdisplacement of an electron from a valence orbit that it occupies in a methane carbon to that of a valence orbit that it occupies in carbon dioxide is thus really a summation of the energy differences evolved in the interdisplacement of the electrons through the various orbits. The farther the electrons are displaced from the carbon atom, and the nearer they are to the oxygen atom, the smaller will be the energy liberated by the combustion of the compound.

5. The pair of electrons held together by two carbon atoms in saturated hydrocarbons may be considered midway between their respective valence shells and the orbit which the electrons occupy in carbon dioxide.

The formula for calculating the gram-molecular heat of combustion of saturated hydrocarbons is

$$\text{Gram-molecular heat of combustion} = 26.05 \times N$$

where N is the number of electrons in the molecule that have the same position around the carbon atom as in methane. The agreement between the values calculated by the above formula and those found experimentally is generally within 0.3 to 0.4 per cent.

In calculating the heat of combustion of alcohols, ethers, and esters postulate 4 above is utilized. Here, owing to the great affinity of oxygen atoms for electrons, the pair of electrons binding the carbon and oxygen atoms in primary alcohols will be in the inner shell of the oxygen atom, close to the normal valence shell, and in the outer shell of the carbon atom. The energy liberated by the combustion of a molecule of this type should therefore be smaller than for the hydrocarbons, which is in accord with the experimental facts. On the other hand, the two displaced electrons in the alcohols do not occupy the

same position with respect to the carbon and oxygen atoms as they occupy in carbon dioxide. They have to be put nearer the oxygen atom. This process liberates heat, and the amount of heat liberated should be the same for all primary alcohols, unless the other groups present in the molecule affect the position of these electrons. The heat liberated in the case of a primary alcohol group amounts to 13 Cal. Therefore, for a primary alcohol, the heat of combustion equals 26.05 $\times N +$ 13 Cal. Similarly, for a secondary alcohol, the correction is +6.5 Cal., and for polyhydric alcohols the heat of combustion equals $26.05 \times N + 13\,a + 6.5\,b$, where a is the number of primary and b the number of secondary alcohol groups. The correction for an aldehyde group (c) is +13, and for a ketone group (d) +6.5.

The following examples serve to show the method of calculation:

Mannitol, $C_6H_{14}O_6$, containing 26 electrons, 2 primary (a), and 4 secondary alcohol (b) groups. The above formula, $26.05\,N + 13\,a + 6.5\,b$, gives $26.05 \times 26 + 13 \times 2 + 6.5 \times 4 = 729.3$, against 727.6 found by experiment.

Glucose, $C_6H_{12}O_6$, containing 24 electrons, 1 primary (a), 4 secondary alcohol groups (b), and one aldehyde group (c). The sum for all equals $26.05 \times 24 + 13 \times 1 + 6.5 \times 4 + 13 \times 1 = 677.2$, against 673.0 found by experiment.

To the RCH—O—R_1 linkage in disaccharides Kharasch assigns the value of 19.5 Cal., but no calculated figures for the heat of combustion of these carbohydrates are listed by him.

OSMOTIC PRESSURE AND RELATED PHYSICAL CONSTANTS, AND THEIR APPLICATION IN DETERMINING MOLECULAR WEIGHTS OF SUGARS

The determination of the molecular weights of sugars and sugar derivatives is a problem which may confront the chemist in his examination of unknown carbohydrates of plant or animal origin.

With a reducing sugar an elementary analysis of one of its osazones or hydrazones (p. 702) will serve to fix the class to which the sugar belongs and thus indicate the molecular weight. However, for non-reducing sugars, such as sucrose, raffinose, etc., and for the sugar derivatives, which do not form osazones and hydrazones, a determination of the molecular weight by some physical method is usually required.

The molecular weights of sugar derivatives, which can be distilled without decomposition or dissociation, are best determined by the well-known vapor-density method of Victor Meyer. All the sugars, however, and most of their compounds undergo decomposition at or below

the melting point so that the vapor-density method is excluded. Recourse, therefore, is usually made to some one of the methods which involve the principle of osmotic pressure.

Osmotic Pressure of Sugar Solutions

Pfeffer,[34] the plant physiologist, in 1877, during his classical studies upon osmosis in vegetable cells, discovered that the osmotic pressure of dilute sugar solutions was proportional to the concentration. Pfeffer's experiments were performed by placing the sugar solutions in a porous bulb, which had deposited within its walls a semipermeable membrane of copper ferrocyanide. The bulb, which was connected with an upright tube, was then immersed in distilled water. The membrane, which is permeable to water but not to sugar, allows water to enter the bulb; the sugar solution begins to rise in the tube, the elevation continuing until, after many hours, a maximum is reached; at this point the difference between the level of liquids within and without the bulb gives a pressure corresponding to the osmotic pressure of the sugar solution. This maximum pressure, expressed in centimeters or millimeters of mercury, was called by Pfeffer the osmotic pressure.

The following results by Pfeffer give the osmotic pressure of sucrose solutions at different concentrations.

Concentration (C) of Sucrose Solution	Pressure (P) in Centimeters of Mercury	Ratio $\frac{P}{C}$
per cent		
1	53.5	53.5
2	101.6	50.8
4	208.2	52.1
6	307.5	51.3

The ratio P/C is a constant, the slight differences noted being due to variations in temperature and other experimental errors.

Pfeffer also showed that the osmotic pressure of sugar solutions underwent a regular increase with elevation of temperature. The following experiment was made upon a 1 per cent sucrose solution.

Temperature °C.	Absolute Temperature (T)	Osmotic Pressure (P)	Ratio $\frac{P}{T}$
14.15	287.15	51.0	0.1776
15.5	288.5	52.05	0.1804
32.0	305.0	54.4	0.1784
36.0	309.0	56.7	0.1835

[34] Pfeffer's "Osmotische Untersuchungen," Leipzig, 1877.

The ratio P/T is thus also found to be constant, the slight variations being due as before to experimental errors.

Relation of Osmotic to Gas Pressure. In 1887 van't Hoff[35] showed that Pfeffer's osmotic pressures were identical in value with those obtained by gas pressure, in other words, that the osmotic pressure per gram molecule of substance is the same as the gas pressure per gram molecule at the same temperature and volume. This identity is expressed by the equation

$$pv = RT$$

in which p is the pressure and v the volume, T the absolute temperature and R a constant. Van't Hoff showed that the constant R is the same for substances in dilute solution as well as in the gaseous state.

The molecular weight of a substance is equal to the weight of its vapor in grams which would occupy the same volume, under equal temperature and pressure, as 2.016 g. of hydrogen (2.016 g. being the weight of the hydrogen molecule). This volume, called the gram-molecular volume, is 22411.5 ml. at 0° C. (273.1° abs.) and 76.0 cm. of mercury pressure (1 atmosphere).

Calling V the volume occupied by a gram molecule of gas we obtain from the previous equation

$$R = \frac{pV}{T}$$

The pressure p, per square centimeter of mercury (sp. gr. = 13.595), and at an acceleration of gravity of 0.980665, is equal to 76 cm. $\times$ 13.595 $\times$ 0.980665 = 1013.2 g. We obtain, therefore, for the constant R,

$$R = \frac{1013.2 \times 22411.5}{273.1} = 83{,}150$$

To prove the identity of this constant for the osmotic pressure of sucrose one of the experiments of Pfeffer may be selected. A 1 per cent solution of sucrose at 0° C. (273.1° abs.) gave an osmotic pressure of 49.3 cm. of mercury. The latter corresponds to a pressure per square centimeter of 49.3 $\times$ 13.595 $\times$ 0.980665 = 657.3 g. Since the molecular weight of sucrose is 342, the volume (V) of a 1 per cent solution containing a gram molecule would be very closely 34,200 ml. Substituting these volumes in the equation, we obtain,

$$R = \frac{657.3 \times 34{,}200}{273.1} = 82{,}310$$

[35] Ostwald's "Grundriss der allgemeinen Chemie," 2nd ed., p. 131.

which value is in substantial agreement with that derived by the other method.

Application of the Method. If we accept now the identity of the laws for gaseous and osmotic pressure, the molecular weight of a sugar can be determined from its osmotic pressure in a manner analogous to that followed by the vapor-density method.

Example. In one of the experiments previously cited Pfeffer found at 15.5° C. (288.6° abs.) for a 1 per cent sucrose solution an osmotic pressure of 52.05 cm. mercury. If 1 g. of sucrose occupies 100 ml. at 52.05 cm. pressure and 15.5° C., then the number of grams which would occupy 22411.5 ml. at 0° C. (273.1° abs.) and 76 cm. pressure would be:

$$\frac{1 \times 22411.5 \times 288.6 \times 76}{100 \text{ ml.} \times 273.1 \times 52.05} = 345.8$$

Thus 345.8, the number of grams in the gram-molecular volume, is the molecular weight of sucrose. This agrees closely with the actual value 342 calculated from the formula $C_{12}H_{22}O_{11}$.

It follows from the previous discussion that the sugars of lowest molecular weight will show for equal concentration and temperature the highest osmotic pressure.

Improved instruments and procedures for measuring osmotic pressure have been described by Frazer and Myrick,[36] Frazer and Lotz,[37] Eichelberger,[38] Ulmann,[39] Hess and Ulmann,[40] Baldes,[41] and others.

Measurement of Osmotic Pressure by Plasmolysis. A second method of applying the principle just described is due to the Dutch botanist de Vries,[42] who discovered that the plasmolysis, or loosening of the protoplasmic lining of plant cells, offered a simple and reliable means of measuring osmotic pressure. Figure 223 shows the microscopic appearance of a plant cell in sugar solutions of different concentration. In such a cell the thin layer p of protoplasm (the protoplast) acts as a semipermeable membrane. So long as the osmotic pressure of the cell liquid l exceeds or equals that of the surrounding sugar solution s, the protoplast is not affected. When, however, the osmotic pressure of the sugar solution becomes greater than that of the cell liquid there is a diffusion of water outward through the protoplasmic mem-

[36] *J. Am. Chem. Soc.*, 38, 1907 (1916).
[37] *J. Am. Chem. Soc.*, 43, 2501 (1921).
[38] *J. Am. Chem. Soc.*, 53, 2025 (1931).
[39] *Physik. Chem.*, Abt. A, 156, 419 (1931).
[40] *Naturwissenschaften*, 20, 296 (1932).
[41] *J. Sci. Instruments*, 11, 223 (1934).
[42] *Bot. Ztg.*, 46, 229, 393 (1888).

brane. The latter, in consequence of the loss of a part of the cell water, is loosened from the cell wall and contracts, as shown in the figure.

The application of the method may be understood from the following: de Vries found that the hair roots of the frogbit (*Hydrocharis Morsus-ranæ*) showed no plasmolysis in a 7 per cent, but a very pronounced loosening of the protoplast in a 7.1 per cent, sucrose solution. For these particular root hairs under the conditions of the experiment, plasmolysis was produced by a solution containing 0.208 g. mol. of sucrose to 1000 g. of solution (71 g. ÷ 342, the molecular weight of sucrose).

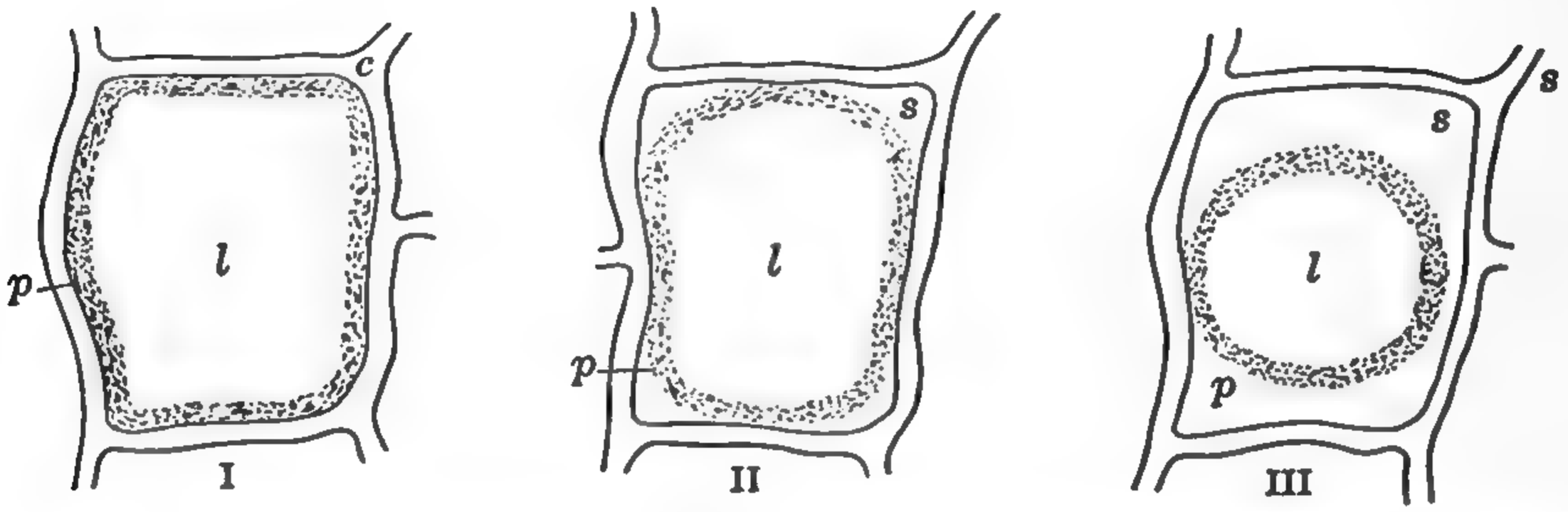


FIG. 223. Illustrating plasmolysis. I. Condition of plant cell before plasmolysis. II. Beginning of plasmolysis. III. Advanced stage of plasmolysis.

Suppose that, using these same root hairs, a solution containing 3.7 per cent of glucose just produced plasmolysis. Then 37 (the grams of glucose per 1000 g. of solution) divided by 0.208 = 178, the molecular weight of glucose, which corresponds to the formula $C_6H_{12}O_6$ (molecular weight = 180).

It was by this means that de Vries,[43] in 1888, established the molecular weight of raffinose. The following formulas had been proposed for the constitution of this sugar:

I. $C_{12}H_{22}O_{11} + 3\,H_2O = 396$, molecular weight.
II. $C_{18}H_{32}O_{16} + 5\,H_2O = 594$, molecular weight.
III. $C_{36}H_{64}O_{32} + 10\,H_2O = 1188$, molecular weight.

De Vries found by his method of plasmolysis that, when standardized against a sucrose solution for the same plant cell, 595.7 parts of raffinose were equimolecular with 342 parts of sucrose. This figure agrees with the molecular weight of formula II; the correctness of de Vries's conclusion was afterwards verified by chemical means.

[43] *Compt. rend.*, 106, 751 (1888).

Owing to the variation in composition of cell liquids, it is evident that the particular plant cells chosen for this method of examination must always be standardized before using.

Freezing and Boiling Points of Sugar Solutions

On account of the difficulty of preparing a perfect semipermeable membrane and owing to the extreme liability of such membranes to rupture, the determination of molecular weights by direct measurement of osmotic pressure, although most sound in principle, is not generally followed. Use is accordingly made of the measurement of some related constant, such as that of vapor pressure, depression of freezing point, or elevation of boiling point. The freezing and boiling points of sugar solutions vary in fact according to their vapor pressure, the value of which, it can be shown, is directly proportional to the osmotic pressure.

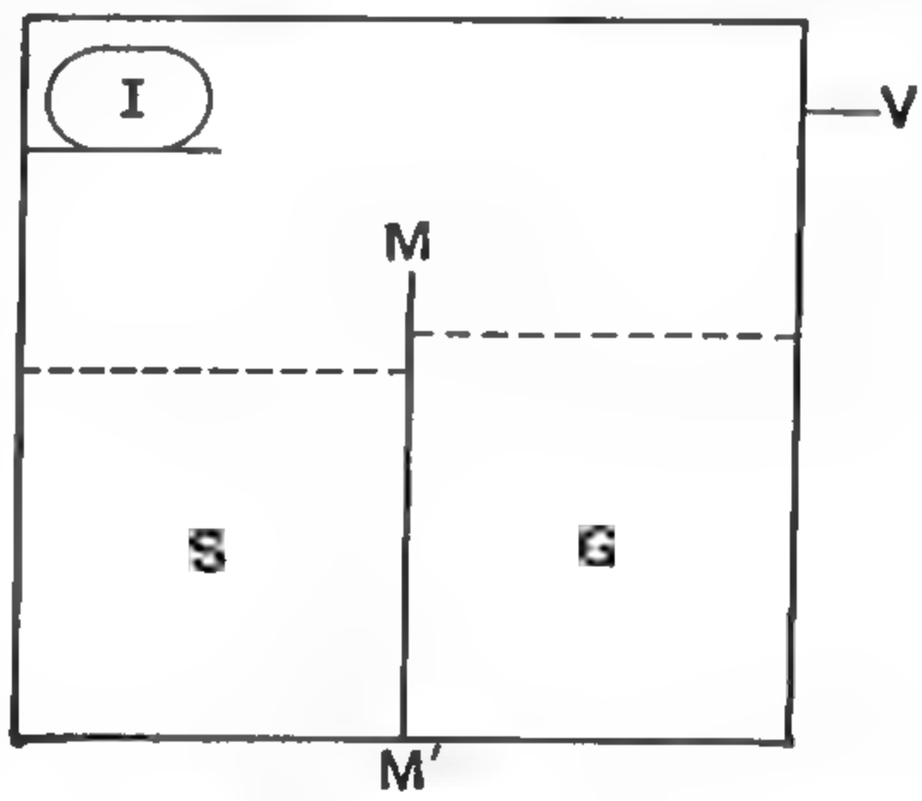


Fig. 224. Illustrating principle of isotonic sugar solutions.

Isotonic Solutions. In Fig. 224 suppose the closed vessel V to be divided by a semipermeable membrane M-M' into two equal compartments, which open into each other above M. Suppose, next, equal volumes of sucrose and glucose solutions of the same concentration to be placed in each of the compartments. Then water will diffuse from the sucrose solution S, where the osmotic pressure is lower, into the glucose solution G, where the osmotic pressure is higher, until at the point of equilibrium the osmotic pressures upon both sides of the membrane are equal. The two sugar solutions are then said to be isotonic and isotonic solutions must have the same vapor pressure. For if the vapor pressures were unequal, water vapor would pass from the solution of higher to that of lower vapor pressure, the concentration of the sugar solutions would thus be changed, and water must again diffuse to the compartment of higher osmotic pressure. There would thus be established a perpetual motion which is contrary to law. Consequently isotonic solutions must have the same vapor pressure.

Suppose next a piece of ice I to be placed in the closed compartment above the partition M, and suppose this ice to be of the same temperature as the freezing point of the isotonic sucrose solution S. Then the vapor pressure between I and S must be equal, otherwise

water vapor would pass between the two and change the freezing point of *S*. But since *S* and *G* are both isotonic and have the same vapor pressure, both must also have the same freezing point.

In the same way the two isotonic solutions *S* and *G* must have the same boiling point, the vapor tension of the aqueous vapor at the boiling point being the same for both solutions.

The proportionality between changes in vapor pressure and between changes in freezing or boiling point is easily illustrated by means of a diagram. In Fig. 225, let *OW* be the pressure curve of water for change in temperature and *OI* the pressure curve of ice, the projection of *O* at *T* being the freezing point of water. Let *Ss* be the corresponding curve of a 1 per cent sucrose solution and *Gg* of a 1 per cent glucose solution, the projection of the points *s* and *g* at *t* and *t'* being the respective freezing points of the two solutions. For comparatively small areas the lines *gO*, *ss'*, and *gg'* may be regarded as straight and *ss'* and *gg'* as parallel. In the $\triangle$ *Ogg'* $Os' : Og' :: Os : Og$ and so also $Os : Og :: Tt : Tt'$. Therefore the lowerings in vapor pressure (and hence osmotic pressure) *Os'* and *Og'* of the two sugar solutions as compared with the solvent water are directly proportional to the corresponding depressions in freezing point *Tt* and *Tt'*.

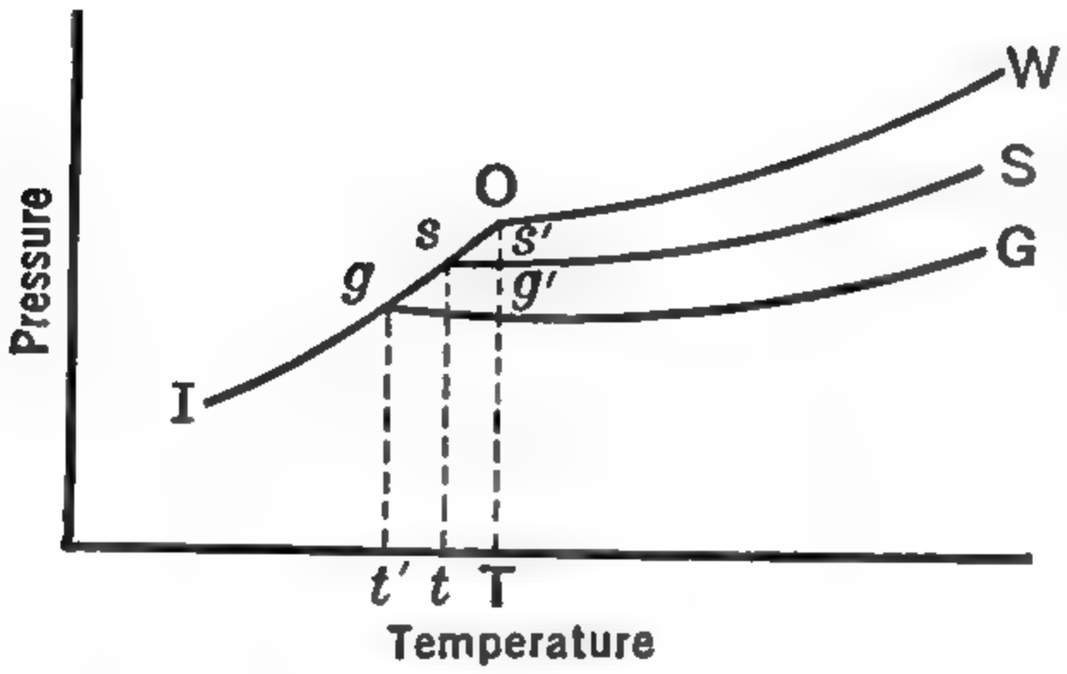


FIG. 225. Showing relation of vapor pressure of sugar solutions to depression of freezing points.

Raoult's Method for Determining Depression of Freezing Point. For determining the depression of freezing points by Raoult's[44] method the apparatus of Beckmann[45] (Fig. 226) is generally used. This consists of a large tube *A* (2.5 cm. by 21 cm.) provided with a side tube *A'*. The main opening is provided with a stopper through which pass the Beckmann thermometer *D* and a small stirrer, provided with a cork handle *r*. The thermometer has a range of about 6°, and the scale is divided into hundredths, the thousandths of a degree being estimated by aid of a magnifying glass. The tube *A* fits through a cork into the larger tube *B*, which serves as an air-jacket, and the whole sits in the cover of a large glass cylinder which is filled with a freezing mixture a few degrees lower than the freezing point of the solution to be examined.

[44] *Compt. rend.*, 94, 1517 (1882); 101, 1056 (1885); 103, 1125 (1886).
[45] *Z. physik. Chem.*, 2, 638 (1888).

In making an experiment, using water as the solvent, the freezing bath is set at about —5° C. and the mercury of the Beckmann thermometer adjusted by means of its regulating device *c*, so that the top of the column falls within the proper range of the scale. A weighed quantity of water, sufficient to cover the bulb of the Beckmann thermometer, is placed in *A*, the thermometer and stirrer are inserted and the tube plunged through the small opening *b* into the freezing mixture. When signs of freezing begin to appear, the tube is withdrawn from the freezing mixture, wiped dry, and then inserted in the air-jacket *B*. The water and forming ice are now stirred vigorously by *r*; the temperature after reaching a certain minimum begins to increase suddenly with the liberation of latent heat. The mercury soon ceases to rise and the point at which it stops, after tapping to prevent any lag, is taken as the freezing point of the water. The operation is repeated several times, the average of the observations being taken as the final value. The same operations are now repeated after introducing through *A'* known weights of the sugar to be examined (1 to 5 g. per 100 g. of water), the maximum point to which the mercury rises after overcooling being taken as the freezing point of the solution. The corrected difference between the freezing point of water and that of water + sugar is the depression of freezing point.

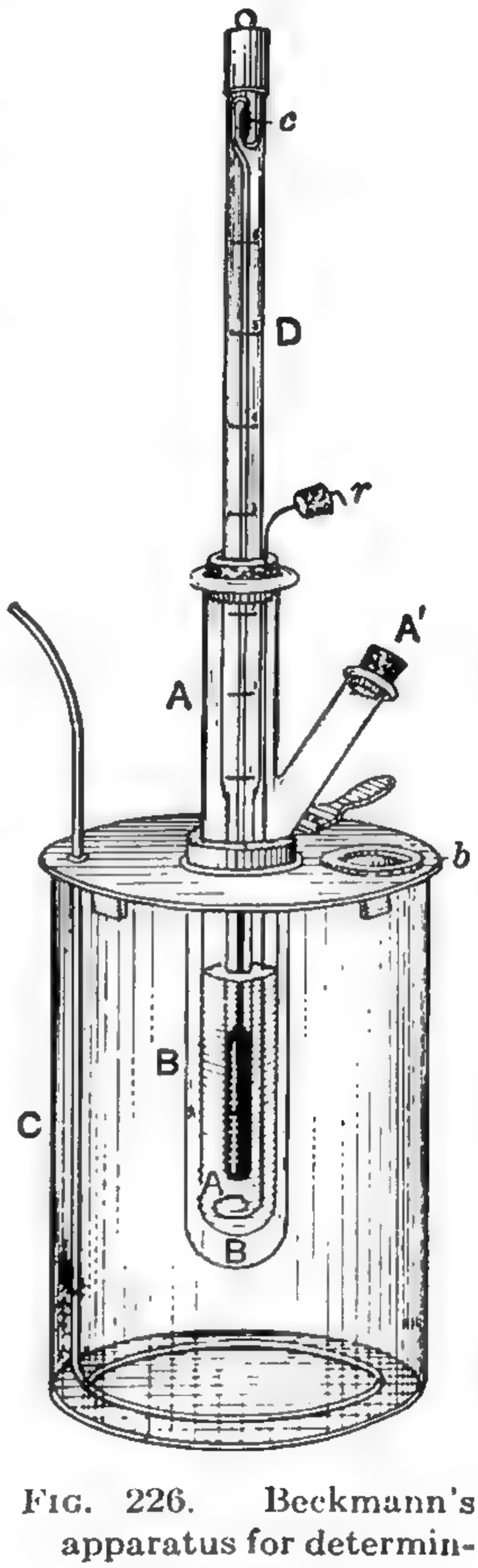


FIG. 226. Beckmann's apparatus for determining depression of freezing point.

Improved models of the Beckmann freezing-point apparatus includes the following accessories: a motor drive for stirrer *r*, a seeding device lowered into the freezing mixture to prepare crystals of the solvent with which the solution in the freezing tube may be inoculated, a thermometer to measure the temperature of the freezing mixture in the outside jar, and a sulfuric acid seal attached to the upper part of the freezing tube to be used for hygroscopic substances. Ordinarily this seal is filled with mercury.

Molecular Depression of Freezing Point. According to what was said under osmotic and vapor pressure, solutions of undissociated substances (non-conducting solutions) which contain the same number of gram molecules per liter should show the same depression of freezing point. The depression for 1 g. mol. of undissociated substance per 1000 g. of solvent, according to van't Hoff,[46] is expressed by the formula $\frac{0.002\,T^2}{W}$, in which T is the absolute temperature of melting, and W the latent heat of melting for the solvent. This expression in case of water, whose latent heat of melting is 80 cal. and temperature of melting 273° abs., would give $\frac{0.002 \times 273^2}{80} = 1.86$. Loomis, as a matter of fact, in the examination of solutions of some 25 different substances obtained a depression in freezing point for 1 g. mol. to 1000 g. of water of almost exactly 1.86° C. The following experiments by Loomis[47] give the results of 6 tests upon maltose. (M, the molecular weight of maltose anhydride $C_{12}H_{22}O_{11} = 342$.)

Grams Maltose to 1000 g. Water (P)	Gram Molecules of Maltose to 1000 g. Water $\left(\frac{P}{M}\right)$	Depression of Freezing Point (Δ), °C.	Molecular Depression of Freezing Point $\left(\Delta \Big/ \frac{P}{M} = \frac{\Delta M}{P}\right)$
3.431	0.0100	0.0193	1.86
6.879	0.0201	0.0378	1.88
10.350	0.0302	0.0560	1.85
17.316	0.0506	0.0946	1.87
35.004	0.1023	0.1919	1.876
71.548	0.2091	0.3946	1.887

Applications of Freezing-Point Method. The application of the freezing-point method to the determination of molecular weights may be understood from the following example:

	Corrected Freezing Point upon Beckmann Scale
20 g. of water in the apparatus gave	4.320°
20 g. of water + 0.3647 g. fructose gave	4.131°
Depression of freezing point (Δ) =	0.189° C.

The grams of fructose calculated to 1000 g. of water would be

$$\frac{0.3647 \times 1000}{20} = 18.235 \text{ g.} = P$$

[46] Ostwald's "Grundriss der allgemeinen Chemie," 2nd ed., p. 142.
[47] *Z. physik. Chem.*, 37, 407 (1901).

Since $$\frac{\Delta M}{P} = \text{the constant } 1.86,\ M = \frac{1.86\ P}{\Delta}$$

Substituting the values obtained for the Δ and P of fructose we obtain

$$M = \frac{1.86 \times 18.235}{0.189} = 179.5$$

which agrees closely with the value 180, required by the formula $C_6H_{12}O_6$.

If w is the weight of sugar taken and W the weight of water, the various steps of the calculation are represented by the general equation:

$$M = \frac{w \times 1000 \times 1.86}{W \times \Delta}$$

The method of determining molecular weight by the depression of freezing point is one that requires considerable care in manipulation, and the inexperienced chemist should thoroughly test the method upon substances of known molecular weight before applying it to the examination of unknown compounds. The method is open to a large number of experimental errors, such as too low a temperature of freezing bath, too high a room temperature, radiation of heat from the observer, faulty thermometer or error in reading, solution of air by the water, careless handling of the instrument, etc. For a thorough discussion of these various points the chemist is referred to the original papers by Raoult, Beckmann, Loomis, and others.[48] Owing to the small value of Δ any slight error in its determination becomes greatly magnified in the final calculation.

The freezing-point method has been successfully employed by Tollens and Mayer, Brown and Morris, and others in determining the molecular weights of many sugars. The following examples of determinations for 9 sugars are selected from a compilation of results by Tollens.[49]

Sugar	Formula	Molecular Weight		Authority
		Calculated	Found	
Arabinose	$C_5H_{10}O_5$	150.08	150.3	Brown and Morris
Xylose	$C_5H_{10}O_5$	150.08	154.1	Tollens and Mayer
Glucose	$C_6H_{12}O_6$	180.10	179	Tollens and Mayer
Invert sugar	$C_6H_{12}O_6$	180.10	174.3	Brown and Morris
Galactose	$C_6H_{12}O_6$	180.10	177	Brown and Morris
Sucrose	$C_{12}H_{22}O_{11}$	342.18	352	Raoult
Maltose	$C_{12}H_{22}O_{11}$	342.18	322	Brown and Morris
Lactose	$C_{12}H_{22}O_{11},H_2O$	360.19	353	Tollens and Mayer
Raffinose	$C_{18}H_{32}O_{16},5H_2O$	594.32	594	Tollens and Mayer

[48] For a complete review and bibliography of the earlier literature see Lippmann's "Chemie der Zuckerarten," 1126; cf. also Adams, *J. Am. Chem. Soc.*, 37, 481 (1915), and Randall and Vanselow, *J. Am. Chem. Soc.*, 46, 2418 (1924).

[49] "Handbuch der Kohlenhydrate," 3rd ed., p. 10.

The freezing-point method can be applied to the examination of sugar solutions for other purposes than those of molecular-weight determination. Kahlenberg, Davis, and Fowler,[50] for example, have employed it in measuring the speed of inversion of sucrose. Table LXXVIII, by the above authorities, gives a comparison of the inversion coefficient of sucrose as determined by the polariscope and freezing-point methods. One-half gram molecule of sucrose to 1000 ml. was inverted at 55.5° C. by 0.01 g. mol. of hydrochloric acid.

TABLE LXXVIII

RATE OF INVERSION OF SUCROSE AS DETERMINED BY POLARISCOPE AND BY DEPRESSION IN FREEZING POINT

Time	Polariscope Reading	Inversion Coefficient K by Polariscope	Depression in Freezing Point	Inversion Coefficient K by Freezing Point
hours			° C.	
0.0	22.62	.....	1.175	.....
1.0	16.58	0.0983	1.393	0.0977
2.0	9.92	0.1205	1.635	0.1217
2.5	7.68	0.1208	1.705	0.1185
3.0	5.94	0.1186	1.809	0.1296
4.0	2.54	0.1215	1.912	0.1263
4.5	1.42	0.1198	1.954	0.1252
7.0	−2.40	0.1130	2.105	0.1254
17.5	−6.90	0.1142	2.230	0.1028
26.5	−7.20	.....	2.247	.....
	Average.....	0.1158		0.1147

It is seen that the value of the constant K, as determined by the Wilhelmy equation $K = \frac{1}{t} \log \frac{a}{a - x}$, is identical by the two methods of measurement.

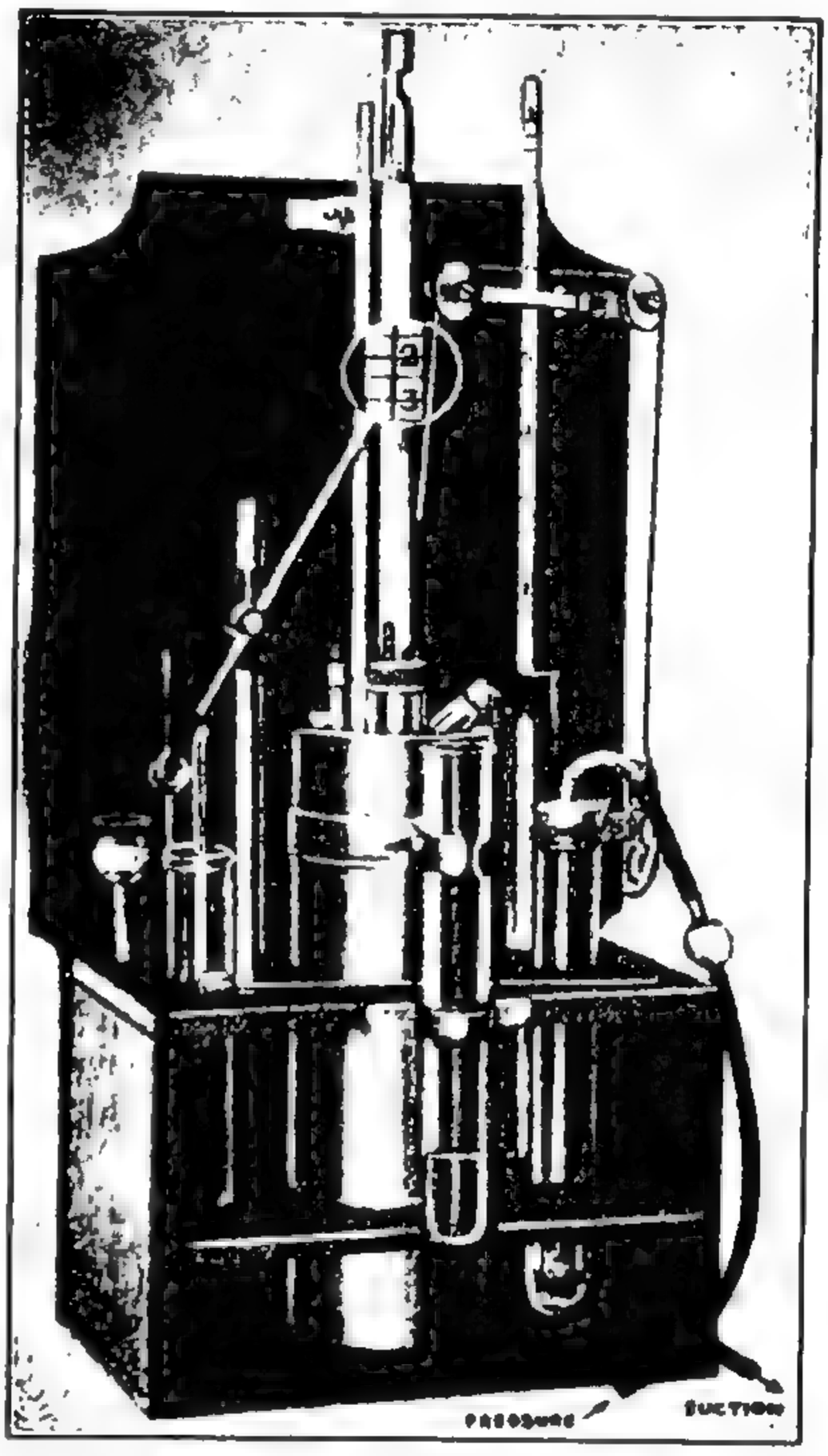
Hortvet's Cryoscope. Hortvet[51] has modified the Raoult-Beckmann apparatus and procedure for the purpose of greater convenience and rapidity of operation in routine work. The instrument was designed especially for determining the amount of added water in milk by measuring the freezing-point depression, but it may be substituted for the Beckmann apparatus in other work also.

The Hortvet cryoscope (Fig. 227) and the method of using it are described in the Methods of Analysis of the Association of Official Agricultural Chemists[52] as follows:

[50] *J. Am. Chem. Soc.*, **21**, 1 (1899).
[51] *J. Ind. Eng. Chem.*, **13**, 198 (1921).
[52] "Methods of Analysis, A. O. A. C.," 5th ed., p. 275, 1940.

APPARATUS

Cryoscope. A cylindrical-shaped Dewar flask of 1-liter capacity and 28-cm. internal depth, surrounded by a metal casing, is tightly closed by means of a large cork of about 3 cm. thickness. Through the center of the cork is tightly fitted a medium thin-walled glass or metal tube, 250 mm. in length by 33 mm. outside diameter. At one side of the cork is inserted a narrow metal inlet tube, the lower end of which is formed into a perforated loop near the bottom of the flask. At the opposite side is a metal tube of T-shape construction and 6 mm. internal diameter, intended to afford escape for vapors, and also for introducing volatile fluid into the apparatus. At the back portion of the cork is fitted a control thermometer, the bulb of which extends nearly to the bottom of the flask. The freezing test tube is of thin glass, about 240 mm. in length by 29 mm. outside diameter, and fits closely into the larger tube, which is sealed into the cork. In the rubber stopper of the freezing tube is fitted the standard thermometer. The length of the thermometer permits insertion of the bulb nearly to the bottom of the tube and at the same time allows complete exposure of the scale above the stopper. At the right side of the thermometer a stirring device made of non-corrodible low-conductivity metal is fitted into the stopper through a short section of thin-walled metal tubing. The lower end extends nearly to the bottom of the test tube and is provided with a horizontal loop encircling the thermometer. At the left of the thermometer is a freezing-starter attachment inserted through an opening in the stopper formed by means of a short section of metal tubing. This device consists of a non-corrodible metal rod, at the lower end of which is a 10-mm. length opening for the purpose of carrying a small fragment of ice. At one side of the cryoscope is installed an air-drying arrangement which consists of a Folin absorption bulb inserted through a tightly fitting stopper and extending nearly to the bottom of a large-sized test tube. A short section of glass tubing is inserted through a second opening in the stopper and is connected to the vaporizing tube which enters the cryoscope. Sulfuric acid is poured into the



(Reproduced with permission from "Methods of Analysis, A.O.A.C.," 5th ed., p. 275.)

FIG. 227. Hortvet cryoscope.

drying tube to a level slightly above the small inner bulb. At the opposite side of the apparatus is arranged a drain tube for the purpose of conducting vapors away from the operator. By means of a pressure and suction pump dry air may be forced into the apparatus at a suitable rate and the mixed vapors conducted out through the base of the drain tube into the sink. An adjustable lens is mounted in a convenient position in front of the thermometer for the purpose of magnifying the scale.

Standard thermometer. A solid-stem instrument having a total length of 58 cm., with a scale portion measuring about 30 cm. The total scale range is 3°, from +1° to −2°, and each degree division is subdivided into tenths and hundredths. The length of a degree division approximates 1 dm., thus making the smallest subdivisions of such magnitudes as to enable easy observation and readings estimated to 0.001°. Standardize the thermometer as directed below. Check at frequent intervals, once a week or as often as may be necessary, to keep an accurate record of any changes that may occur.

Control thermometer. A solid-stem instrument approximately 58 cm. in length and having a scale range of +20° to −30°. Test in a bath of melting crushed ice for the purpose of determining whether the 0 mark on the scale is correct. The scale graduations should be accurate to within 0.10°.

STANDARDIZATION OF THE THERMOMETER

Make 3 freezing-point determinations by the procedure described below on each of the following:

(a) *Recently boiled distilled water.*

(b) *Sucrose solution.* Dissolve 7 g. of pure sucrose in water and make the solution to a volume of 100 ml. at 20°.

(c) *Sucrose solution.* Dissolve 10 g. of pure sucrose in water and make the solution to a volume of 100 ml. at 20°.

(A sample of pure sucrose may be obtained by application to the Director of the Bureau of Standards, Department of Commerce, Washington, D. C.)

Tabulate the results in the following form:

Freezing-Point Observations	Pure Water	7 g. Sucrose Solution		10 g. Sucrose Solution	
		Observed Freezing Point (−*S*)	Freezing-Point Depression *S* − *W* (algebraic)	Observed Freezing Point (−*S*)	Freezing-Point Depression *S* − *W* (algebraic)
1st					
2nd					
3rd					
Averages	±W	xxxxxxx		xxxxxxx	

Express the results as degrees freezing-point depression below the average of the observed freezing points obtained on the sample of pure water (±W)

which may be above (+) or below (—) the 0 mark on the scale. Obtain each freezing-point depression of the sucrose solutions by the algebraic subtraction of the average of the freezing-point readings of pure water (±W) from each observed freezing point.

Omit adventitious results, i.e., results which are in marked disagreement with other results obtained by carefully following instructions.

Apply the average of the freezing-point depressions obtained on the standard sucrose solutions for the purpose of correcting the thermometer readings obtained on the sugar product under examination.

DETERMINATION

Insert the funnel-tube into the vertical portion of the T-tube at one side of the apparatus and pour in 400 ml. of ether previously cooled to 10° or lower. Close the vertical tube by means of a small cork and connect the pressure pump to the inlet tube of the air-drying attachment. Adjust the pump so as to pass air through the apparatus at a moderate rate, as may be judged by the agitation of the sulfuric acid in the drying tube. Continuous vaporization of the ether will cause a lowering of the temperature in the flask, from ordinary room temperature to 0° in from 5 to 10 minutes. Continue the temperature lowering until the control thermometer registers near —3°. At this stage, by lowering the gauge tube into the ether bath, then closing the top by means of the forefinger and raising to a suitable height, an estimate can be made as to the quantity of ether necessary to pour in for the purpose of restoring the 400-ml. volume. When the volume of ether has been adjusted to 400 ml. an additional 10–15 ml. is sufficient on an average for each succeeding determination. Pour into the freezing test tube sufficient water (30–35 ml.), boiled and cooled to 10° or lower, to submerge the thermometer bulb. Insert the thermometer together with the stirrer and lower the test tube into the larger tube. A small quantity of alcohol, sufficient to fill the lower space between the two test tubes, will serve to complete the conduction medium between the freezing bath and the liquid to be tested. Keep the stirrer in steady up-and-down motion at a rate of approximately one stroke each 1 or 2 seconds, or even at a slower rate, providing the cooling proceeds satisfactorily. Maintain a passage of air through the apparatus until the temperature of the cooling bath reaches —2.5°, at which time the top of the mercury thread in the thermometer usually recedes to a position near the freezing point of water. Maintain the temperature of the cooling bath at —2.5° and continue the manipulation of the stirrer until a supercooling of sample of 1.0 to 1.2° is observed. As a rule, at this time the liquid will begin to freeze, as may be noted by the rapid rise of the mercury. Manipulate the stirrer slowly and carefully three or four times as the mercury column approaches its highest point. By means of a suitable light-weight cork mallet tap the upper end of the thermometer cautiously a number of times until the top of the mercury column remains stationary for at least 1 minute. Observe the exact reading on the thermometer scale, taking necessary precautions to avoid parallax,

and estimate to 0.001°. When the observation has been satisfactorily completed, make a duplicate determination; then remove the thermometer and stirrer and empty the water from the freezing tube.

To determine the freezing-point depression for an unknown sugar, prepare an aqueous solution of known percentage by weight. Rinse the tube with about 25 ml. of the solution, cooled to 10° C. or lower; measure into the tube 30–35 ml. of the solution, or enough to submerge the thermometer bulb, and insert the tube into the apparatus. Maintain the temperature of the cooling bath at 2.5° below the probable freezing point of the solution. Make the determination by the same procedure as that used in determining the freezing point of water. As a rule it is necessary, however, to start the freezing action in the solution by inserting the freezing starter (which has been kept in contact with ice for several minutes, and in the open end of which has been wedged a fragment of ice) at the time when the mercury column has receded to 1.0–1.2° below the probable freezing point. A rapid rise of the mercury results almost immediately. Remove the starter and manipulate the stirrer slowly and carefully two or three times while the mercury approaches its highest point. Complete the adjustment of the mercury column in the same manner as in the preceding determination; then, avoiding parallax, observe the exact reading on the thermometer scale and estimate to 0.001°. The algebraic difference between the average of readings obtained on the water and the reading obtained on the solution represents the freezing-point depression of the solution. Apply the necessary correction to the result, on the basis of the freezing-point depressions observed with the standard sucrose solutions. Calculate the molecular weight of the unknown sugar from the corrected freezing-point depression, as shown on p. 532.

Because of the great sensitivity of the freezing-point method, Dixon and Mason[53] made use of it for determining small quantities of sucrose in plant saps, by making measurements before and after inversion with invertase. They found experimentally that the depression after inversion is 2.034 times that before inversion. It is not exactly doubled by the change of one disaccharide molecule into two monosaccharide molecules, because during the inversion water is removed from the solvent and combines chemically with the solute. The determination of the freezing-point depression before inversion is made in the presence of the invertase which at the low temperature used causes no noticeable inversion. The advantage claimed for this method is that no clarification is necessary to prepare the product for analysis.

Beckmann's Method for Determining Elevation of Boiling Point. Beckmann's[54] method of determining molecular weights by the eleva-

[53] *Louisiana Planter*, 64, 397 (1920).

[54] *Z. physik. Chem.*, 3, 603 (1889); 4, 532 (1889); 5, 76 (1890); 6, 437 (1890); 8, 223 (1891).

tion of boiling point is the same in principle as that by depression of freezing point. A gram-molecule solution of an undissociated substance should show according to van't Hoff's formula $0.002\ T^2/W$ (in which $T = 373°$, the absolute boiling point of water, and $W = 536$ cal., the latent heat of evaporation), an elevation in boiling point of $\frac{0.002 \times 373^2}{536} = 0.519° = \Delta$.

Beckmann[55] found in one experiment an elevation in boiling point of 0.315° C. for a solution containing 216.8 g. of sucrose to 1000 g. of water, or $216.8 \div 342 = 0.634$ g. mol. The elevation in boiling point for a 1 g.-mol. solution would then be $0.315 \div 0.634 = 0.497°$ C., which is slightly lower than the value calculated by van't Hoff's formula.

The general formula for calculating molecular weights from the elevation in boiling point (Δ) is similar to the formula for the freezing-point method (p. 533) and is

$$M = \frac{w \times 1000 \times 0.52}{W \times \Delta}$$

The boiling-point method, upon the whole, is open to more sources of error than the freezing-point method and has proved much less satisfactory as a means of establishing the molecular weights of sugars.

According to Beiser and Pringsheim[56] very dilute solutions of carbohydrates, including sucrose, cause a depression of the boiling point of water, owing probably to a colloidal condition, and correct molecular-weight results are obtained only at concentrations beyond a certain minimum value.

SURFACE TENSION

Surface tension is defined as the force that impels a liquid to assume the shape presenting the smallest possible surface area, which shape is that of a sphere. It is caused by molecular attraction, which, on the surface of a liquid in a vessel, can act only sideways and downward, but not upward. Strictly speaking, the surface tension is the interfacial tension between the liquid and the vapor phase directly above it, but in practice the surface tension measured is that between the liquid surface and the vapor and air phase. For water and aqueous solutions the error due to this cause is very small and may be neglected.

The unit of surface tension is the dyne per centimeter, which is equivalent to the erg per square centimeter. Surface tension varies

[55] *Z. physik. Chem.*, **6**, 459 (1890).
[56] *Ber.*, **66B**, 1296 (1933).

with the temperature, and for comparative purposes measurements should always be made at a standard temperature.

Surface-Inactive and Surface-Active Substances. It has been observed that many substances when dissolved in water have very little effect on the surface tension unless the solute concentration is very high, whereas others depress it considerably even at very low concentrations. The first group, called surface-inactive, comprises inorganic salts and polyhydroxy compounds like the sugars. Some of these decrease, others increase, the surface tension to a slight extent. To the second group, called surface-active, belong many organic compounds, such as alcohols, aldehydes, acids, esters, etc., and particularly also many hydrophilic colloids, like proteins, gums, soaps, saponins, and others.

Dynamic and Static Surface Tension. When a substance is dissolved in water or some other solvent, the solution thoroughly agitated, and the surface tension measured as soon as the liquid comes to rest, a different value is usually obtained from that found after the solution has been standing for some time. An adsorption equilibrium is gradually established and the surface tension assumes a constant value, which is termed the static surface tension. The initial and intermediate values represent the dynamic surface tension, which consequently is a function of the elapsed time, while the static surface tension represents a definite final constant. For pure liquid compounds the static surface tension is, of course, identical with the dynamic.

Solutes showing positive adsorption tend to accumulate in the surface, and the surface tension decreases during standing. Solutes showing negative adsorption migrate downward into the body of the solution, and the surface tension increases during standing. Sucrose is an example of this class. Solutions in which the dissolved substance is molecularly dispersed reach the static equilibrium rapidly, usually in a few seconds, but hydrophilic colloids require a much longer time, sometimes hours. With solutions of the latter class, measurements of dynamic surface tension are of little value and may even be misleading; comparisons should always be made only on the basis of the static surface tension.

A number of methods may be employed for the measurement of surface tension.[57] Only three of them will be discussed here, the capillary-rise method, which is the simplest theoretically, and two procedures which have been used more particularly for sugar products, the stalagmometer method of Traube, and the ring method of du Noüy.

[57] Freundlich, "Kapillarchemie," 3rd ed., pp. 17–35, 1923.

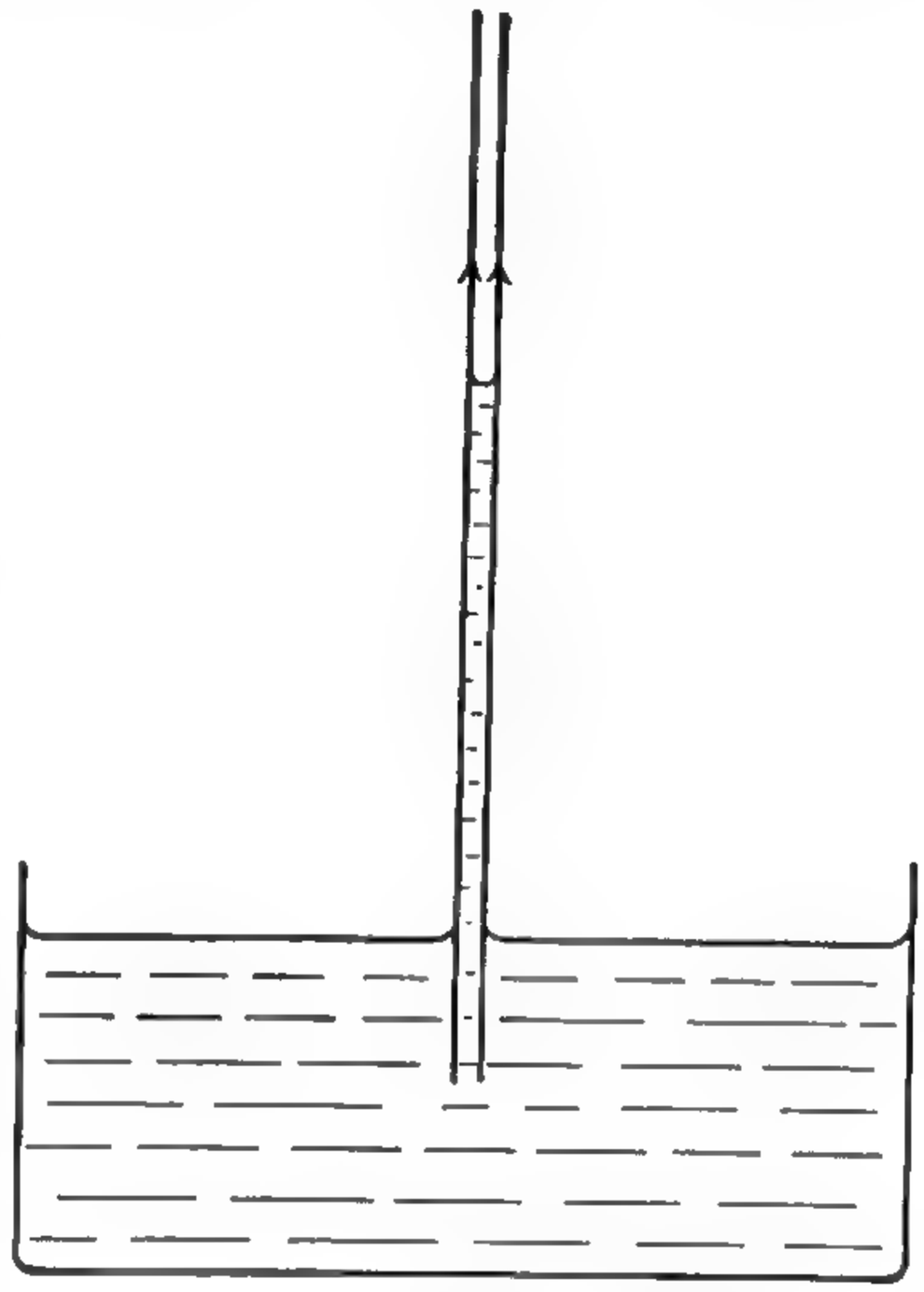
Capillary-Rise Method. In Fig. 228, a capillary tube is partly immersed vertically in a liquid which wets glass, e.g., water. Owing to surface tension, the water rises in the capillary until equilibrium with the force of gravity is reached. If γ is the surface tension, and r the radius of the capillary, the total lifting force acting on the liquid in the capillary is $2r\pi\gamma$. The weight of the liquid lifted equals $r^2\pi hdg$, where h is the height of the column, d the density of the liquid, and g the gravitation constant. Then

$$2r\pi\gamma = r^2\pi hdg$$

and

$$\gamma = \frac{rhdg}{2}$$

If h and r are expressed in centimeters, γ is obtained directly in dynes per centimeter. Both h and r must be measured with high precision, corrections must be applied for the meniscus in the capillary and that in the outer vessel, and d must be corrected for the density of the air-vapor mixture above the surface.[58] Because of these rigid requirements the method is not used much in practice.



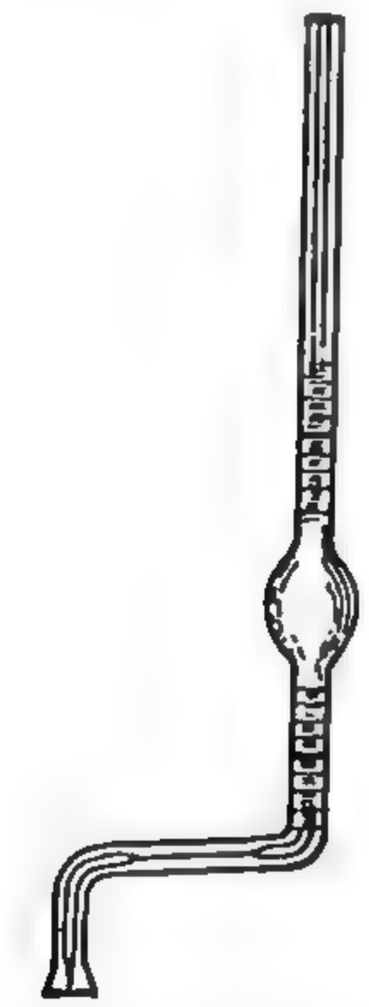
(Reproduced with permission from Freundlich, "Kapillarchemie," p. 25.)

FIG. 228. Showing principle of capillary-rise method for measuring surface tension.

Traube's Stalagmometer.[59] This apparatus, which is extensively used because of its easy manipulation, is shown in Fig. 229. It consists of a vertical glass tube with a bulb holding a known volume of liquid between two marks. At the lower end a horizontal piece of capillary tubing is attached, which bends downward again at a right angle, and ends in a carefully ground tip with a flattened edge. The apparatus is filled with the liquid to be measured, and the number of drops emerging is counted, from the time the liquid passes the upper mark until it reaches the lower mark. According to the law of Tate the drop weight is directly proportional to the surface tension and to the cross section of the capillary outlet. This law is only a first approximation, and if absolute values are desired various corrections must be applied, as

[58] Richards and Carver, *J. Am. Chem. Soc.*, 43, 827 (1921).
[59] *Ber.*, 20, 2644 (1887).

shown by Harkins and Brown.[60] It is possible, however, to calibrate a given instrument with a liquid of known surface tension. If the density and viscosity of the solution to be measured do not differ widely from those of the standard, the ratio between the surface tensions is practically the same as that of the drop weights. The stalagmometer furnishes dynamic values, unless the time allowed for the drops to form is sufficiently long for equilibrium to be established. For practical reasons the time must be restricted, however, and Tödt[61] recommends the choice of a capillary that will deliver not more than 20 drops per minute. If the capillary is standardized with water ($\gamma = 72.8$ dynes per centimeter at 20° C.), and the measurements are made at 20° C., the surface tension of the solution examined is found from the following equation, based on the law of Tate:



(*Courtesy of Eimer and Amend.*)

FIG. 229. Traube stalagmometer.

$$\gamma = \frac{72.8\, d n_w}{n} \text{ dynes per centimeter}$$

where n_w is the number of drops of water, n the same for the unknown solution, and d the density of the latter. The precision of the measurements is increased by means of scales engraved above and below the bulb marks, which make it possible to estimate fractions of a drop. The orifice of the capillary must be perfectly clean, so that it is completely wetted by the liquid. Air bubbles in the liquid must be avoided, and the room must be free from vibrations which would cause the drops to fall prematurely.

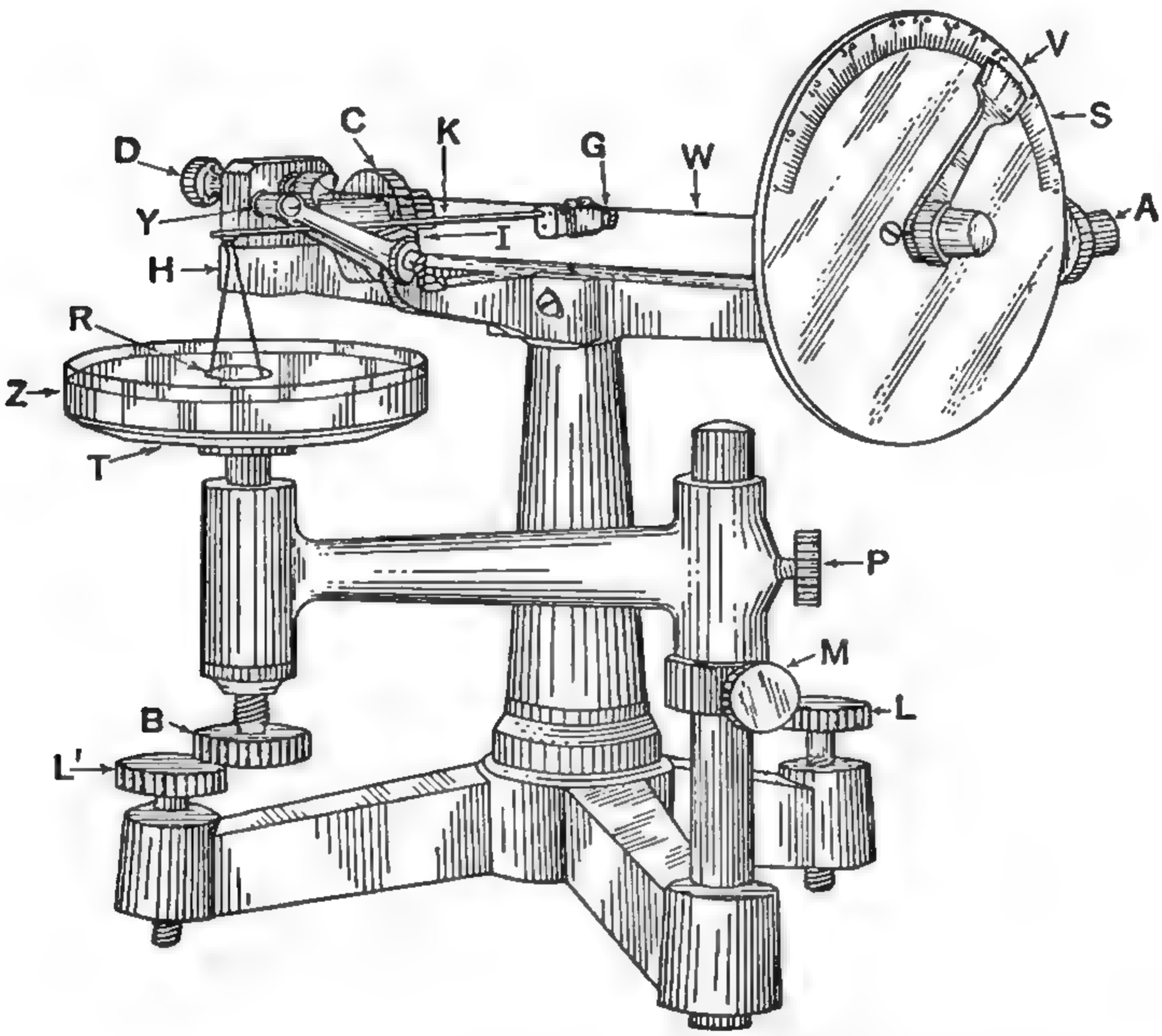
Ring Method of Du Noüy. According to Lecomte du Noüy[62] the methods based on capillary rise or on the weight of hanging drops are unsatisfactory for measuring the surface tension of colloid solutions, and only the so-called ring method is suitable for this purpose. In this method the downward pull exerted on a ring just in contact with the surface of a liquid is determined by means of a counterbalancing upward force. This may be applied by suspending the ring on one beam of a balance and placing weights on the other beam, or by the simpler principle of the torsion balance. The surface tension is calculated by the formula

$$\gamma = \frac{Mg}{2L}$$

[60] *J. Am. Chem. Soc.*, 38, 246 (1916); 41, 499 (1919).
[61] *Z. Ver. deut. Zucker-Ind.*, 76, 253 (1926).
[62] "Surface Equilibria of Biological and Organic Colloids," 1926.

where M is the weight equalizing the pull on the ring, in grams; g the gravity constant; and L the mean circumference of the ring. L is doubled because the pull is caused by two films, one inside and one outside of the ring.

Du Noüy Precision Tensiometer.[63] This instrument is illustrated in Fig. 230. The ring R, 4 cm. in circumference and made of platinum-iridium, is suspended by a stirrup at one end of the horizontal beam K, the other end of which is attached at a right angle to the torsion wire W.



(*Courtesy of Central Scientific Co.*)

FIG. 230. Du Noüy precision tensiometer.

One end of the wire is fixed, while a torque may be applied to the other end by means of a lever with pointer, moving over a circular scale. To calibrate the instrument, it is placed on a table at such a height that the wire is about in line with the eye, and is leveled by screws L and L'. The torsion wire is stretched taut by turning knurled head D. The nut G is adjusted so as to lengthen beam K to about half of the maximum extension provided by that adjustment. Screw A is turned until the

[63] The description of the du Noüy instruments and their use is taken from *Bull.* 101 of the Central Scientific Co., Chicago, Ill.

vernier reads exactly 0. The ring *R* is hung on the free end of beam *K*, and a small strip of paper is placed on the ring. The torsion screw *C* is next turned until the index *I* is exactly opposite the reference line on the mirror. This compensates for the weight of the paper. The apparatus is now ready for the actual calibration of the scale. A known weight, between 500 and 800 mg., is placed on the paper platform. Supposing that a weight of 600 mg. is used, and the circumference of the ring is 4 cm., then according to the formula given above the corresponding surface tension

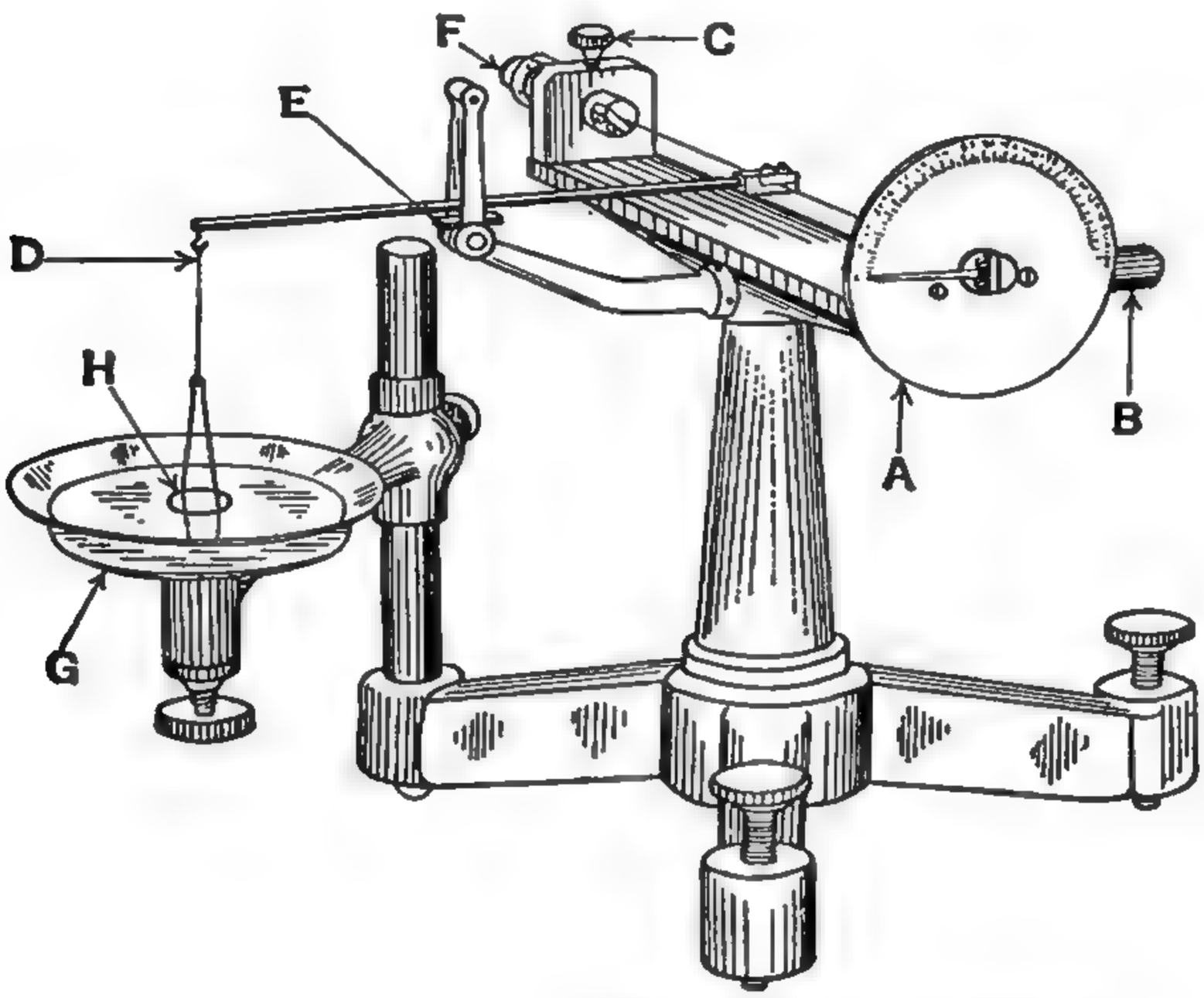
$$\gamma = \frac{0.600 \times 980.7}{2 \times 4} = 73.55 \text{ dynes per cm.}$$

For the gravity constant, the proper value for the place where the instrument is being used is inserted. With the weight on the paper platform, the adjustment head *A* is turned until the index *I* is again exactly opposite the reference line on the mirror, and the dial is read by means of the vernier to 0.1 scale division (0.05 division may be estimated). If the reading is higher than the value as calculated above, screw *G* is turned so as to shorten beam *K*; if the reading is lower, *G* is turned so as to lengthen it. This calibration procedure is repeated readjusting the 0 position after each change of *G*, until the correct dial reading is obtained. Finally the paper on the ring is removed, and with the vernier on the pointer at 0, screw *C* is readjusted so that the beam is again in the 0 position. The instrument is now ready for measurements of surface tension in absolute units.

The simplified apparatus, Fig. 231, does not read directly in dynes, but has an arbitrary scale. It is useful for many purposes where highest precision is not required. The calibration procedure is similar to, but simpler than, that prescribed for the precision instrument. After the torsion wire has been tightened, the pointer on the scale is set at 0 by means of knurled head *B*. The ring is suspended from its hook, set screw *C* is loosened, and the beam is placed in position by turning the adjustment nut *F* until it just clears the small platform *E*. Screw *C* is tightened again. The pointer is now set at a definite point on the scale, say 120; a small piece of paper, the weight of which has been accurately determined, is placed on the ring; and enough weights are added to the paper until the beam returns to the 0 position. The dynes per centimeter corresponding to the chosen point on the scale are now calculated from the total weight, including the paper, placed on the ring, by the formula given above in discussing the calibration of the precision instrument. The dynes per centimeter for other points

on the scale are directly proportional to those for the point used in the calibration.

Measurement of Surface Tension with Du Noüy Tensiometer. All the glassware used for surface-tension measurements should be boiled for 2 hours in chromic acid mixture containing 10 to 15 per cent potassium dichromate, and the objects should be moved around in the acid to expose all surfaces and to remove air bubbles. They are then thoroughly rinsed with a jet of distilled water, and dried on filter paper in



(*Courtesy of Central Scientific Co.*)

FIG. 231. Du Noüy simple tensiometer.

a place free from dust, such as an incubator. If watch glasses or Petri dishes are used as containers for the liquids to be measured they may be flamed over a Bunsen burner to make them completely wettable. The ring must also be flamed before each determination. After the instrument has been leveled and calibrated, with the ring system in its 0 position and the scale at 0, the vessel with the liquid to be measured is placed on the adjustable platform, concentrically below the dry ring. The platform is slowly raised until the liquid surface just touches the ring. It is now lowered very slowly, and at the same time the torque on the wire is increased by turning the pointer in such a way that the beam remains exactly in the 0 position. When the breaking point is approached the adjustment is made still more slowly. At the moment when the ring breaks away, the beam still being in the 0 position, the

surface tension, at the particular temperature used, is read on the scale, directly in dynes per centimeter if the precision instrument is employed, or in an arbitrary scale on the simplified apparatus. In the latter case the surface tension is computed from the calibration value, proportionally to the scale readings.

If absolute surface-tension values are not required, and it is desired merely to record differences in surface tension, the operations may be simplified and speeded up. In this case it is sufficient to raise the platform until it makes contact with the ring, the beam being in the 0 position, and then to increase the torque gradually, without readjusting the 0, until the ring breaks away. The values obtained in this manner are slightly higher than the absolute figures, but the differences between any two readings are the same as those between the absolute values.

Determination of the Correction Factor. A complete theory of the ring method has been developed by Freud and Freud[64] and has been confirmed experimentally by Harkins and Jordan.[65] It was found that the simple formula given on p. 542 does not hold strictly, but that the expression $Mg/2L$ must be multiplied by a factor F which is a complex function of the mean radius R of the ring, the radius r of the wire of the ring, and the maximum volume V of liquid elevated above the surface of the liquid. F may be found from the values of R/r and of R^3/V, in the tables given by Harkins and Jordan. The values of R and r may be calculated from the dimensions of the ring, furnished by the manufacturers, or measured with a screw micrometer, as described by Harkins and Jordan. V equals $W/(D - d)$, where W is the weight of the liquid raised above the surface, D the density of the liquid, and d the density of air saturated with the vapor of the liquid. W may be computed directly from the scale readings on the basis of the calibration data, or may be determined, after a measurement is completed, by adding known weights to the platinum ring to bring the torsion arm back to its 0 position. The value of d is obtained from tables. In the case of water vapor it may be determined from the formula:

$$d = \frac{d_1(H - 0.378\,P)}{H}$$

where d_1 is the density of dry air, H the barometric pressure in millimeters, and P the vapor pressure of water in millimeters, at the given temperature.

For the 4-cm. ring of the du Noüy instrument the factor F equals

[64] *J. Am. Chem. Soc.*, **52**, 1772 (1930).
[65] *J. Am. Chem. Soc.*, **52**, 1751 (1930).

about 0.990 at room temperature for water. This value may be used in determinations on liquids whose surface tension is close to that of water, but in precision measurements F should be determined for each particular set of conditions.

With the du Noüy apparatus it is possible to determine the surface tension at any time after the solution has been made up, from the moment when it has become homogeneous after vigorous stirring (dynamic value), until the adsorption equilibrium has been fully established (static value).

Surface Tension of Sugar Solutions. The surface tension of sucrose solutions has been measured by a number of investigators, but the data found are widely divergent, partly because of the unreliability of the methods used, and partly because most of the values reported represent dynamic values determined after varying time intervals. Measurements of the static surface tension of solutions up to 60 per cent concentration, at 20° C., have been made by Smoleński and Kozlowski[66] with the du Noüy precision tensiometer. The results, which were not corrected by the factor F, may be expressed by the following formula:

$$\gamma = 73.0 + 0.089\ c \text{ dynes per cm.}$$

where c is the sucrose concentration. According to the same authors the static surface tension of a 22.6 per cent solution decreases 0.13 dyne per cm. for each degree Centigrade increase in temperature.

Landt[67] has determined the dynamic surface tension of sucrose, glucose, fructose, galactose, and maltose, at 20° C., with the stalagmometer of Traube. His results are as follows:

	Concentration, Per Cent by Weight	Surface Tension, dynes per cm.
Sucrose	10.2	73.39
Sucrose	20.0	74.16
Sucrose	29.0	74.82
Glucose (anhydrous)	10.5	73.75
Glucose (anhydrous)	19.7	74.71
Glucose (anhydrous)	29.4	75.86
Fructose	9.7	73.60
Fructose	20.6	74.64
Fructose	29.8	75.44
Galactose	10 (about)	73.57
Galactose	20 (about)	74.59
Maltose hydrate	7.5	73.24
Maltose hydrate	15.1	74.04
Maltose hydrate	22.8	74.57

[66] *Gaz. Cukrownicza*, 67, 669 (1930).

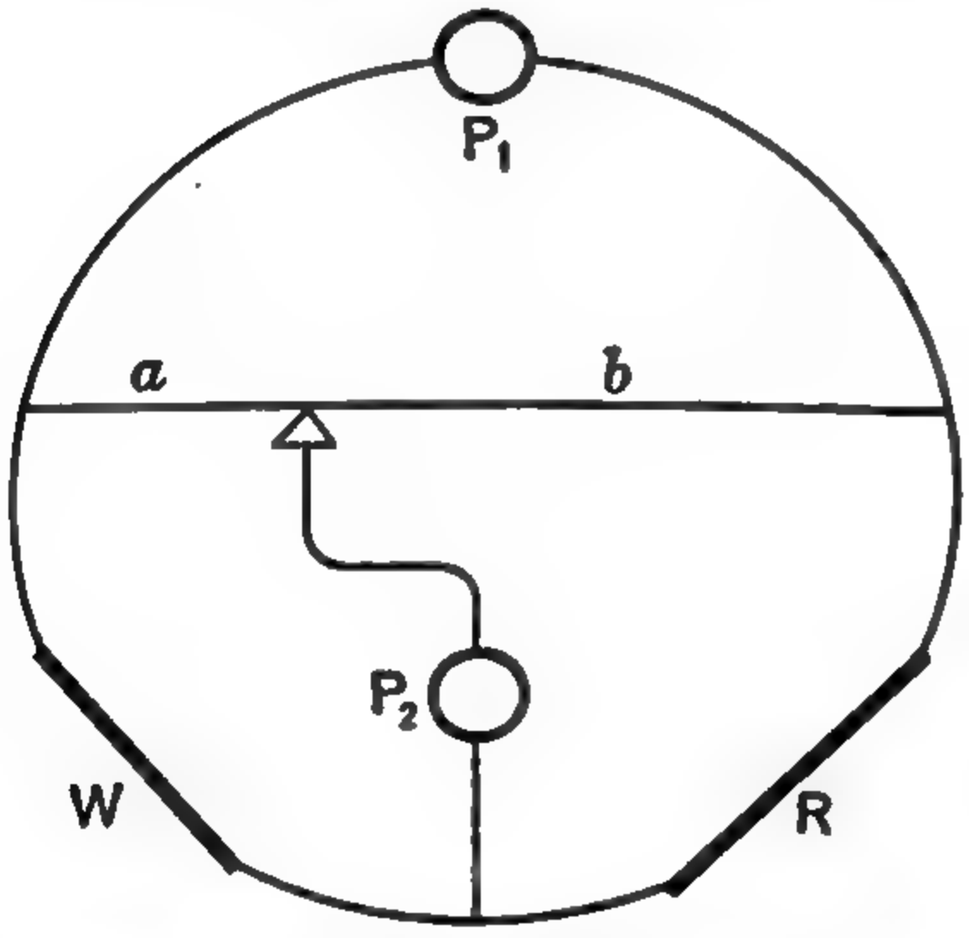
[67] *Z.Ver. deut. Zucker-Ind.*, 81, 119 (1931).

Water, under the same experimental conditions, had a surface tension of 72.68 dynes per cm. It is noted that all the sugars tested by Landt increase the surface tension of water and hence are surface inactive. The effect increases with the concentration of each sugar.

The surface tension of technical sugar products is discussed in Chapter XVII.

ELECTRICAL CONDUCTIVITY OF SUGAR PRODUCTS

Electrical-conductivity measurements are used extensively in the laboratory for analytical purposes, and also for the control of technical operations in the plant. They serve to detect and measure the quantity of ionized salts present as impurities in sugar products, to study and control diffusion, saturation, pan and centrifugal work, and the composition of the various feed, diffusion, wash, and waste waters. In sugar solutions of high concentration the conductivity becomes an indirect measure of the viscosity, and hence of the supersaturation. Solutions of pure sugars not only are very poor conductors of electricity, but also depress the conductivity of electrolytes. This property is also made use of in sugar analysis. The methods for measuring electrical conductivity will be described here, but their application to the analysis of sugar products is reserved for Chapter XVII.



(Reproduced with permission from Kohlrausch-Holborn, "Leitvermögen der Elektrolyte," p. 38.)

FIG. 232. Showing principle of Kohlrausch's method for measuring conductance.

Measurement of Conductivity. In actual practice the resistivity, which is the reciprocal of the conductivity, is measured generally by the Kohlrausch method.

The principle of the method is explained by the diagram, Fig. 232. *R* represents a coil the resistance of which is known; *W* is the conductor the resistance of which is to be measured; P_1 is a source of alternating current; P_2 a null indicating instrument, usually a telephone receiver or a galvanometer; *a*–*b* is a wire equipped with a measuring scale. A sliding contact may be moved over the entire length of the wire. When the contact is set at a point where no current flows through the system, as indicated by practical disappearance of sound in the telephone, then the resistance of *W* is to the known

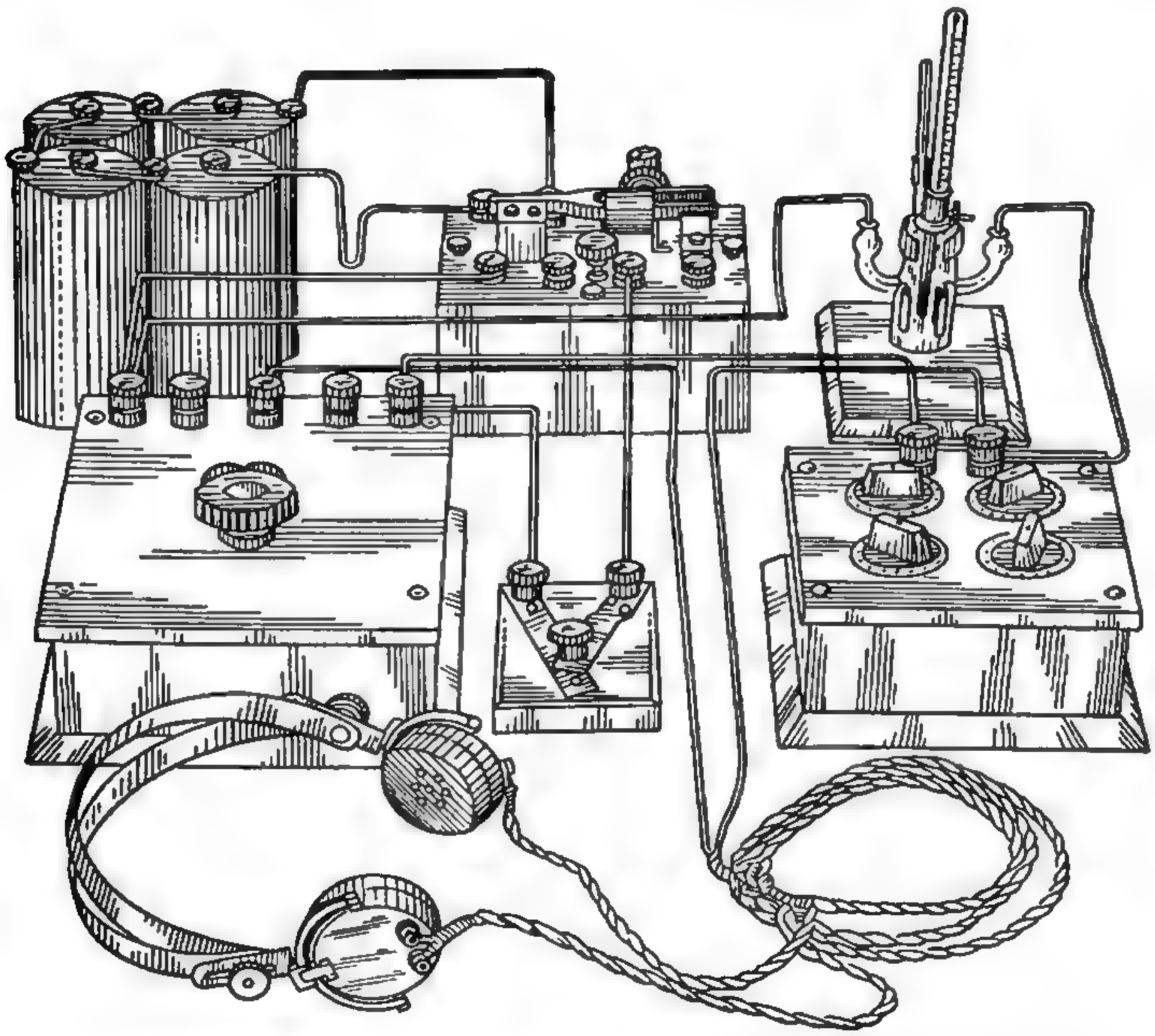
resistance R as a is to b. Hence

$$W = \frac{R \times a}{b}$$

The scale of the slide wire is usually divided into 1000 equal parts, and then

$$W = \frac{R \times a}{1000 - a} \tag{1}$$

A simple assembly, well suited for the precision required in conductivity determinations on sugar products, is shown in Fig. 233. The direct current furnished by the dry cells is converted into high-frequency



(*Courtesy of Leeds and Northrup Co.*)

FIG. 233. Apparatus assembly for measuring specific conductance.

alternating current by means of a microphone hummer. The resistance box is of the four-dial type, and can be set at any value from 1 to 9999 ohms. The slide wire is of the circular form, and enclosed in the box at the lower left of the picture. The greatest precision is obtained when the known and unknown resistances are nearly equal, i.e., when the scale reading is near 500. In the equipment shown the precision can be in-

creased tenfold by inserting at both ends of the slide wire a coil having a resistance 4.5 times that of the slide wire. When these extension coils are used, by making connection through the outer instead of the inner pair of binding posts on the slide-wire box, then

$$W = \frac{4500 + a}{5500 - a} \qquad (2)$$

The circuit should be closed no longer than is necessary, in order to avoid heating the solution. For this reason a tapping key is provided. The microphone hummer should be placed on a felt pad and covered with a bell jar so that the humming noise may not interfere with the detection of the sound minimum in the telephone receiver, which is preferably of the tunable type, to adjust the sensitiveness.

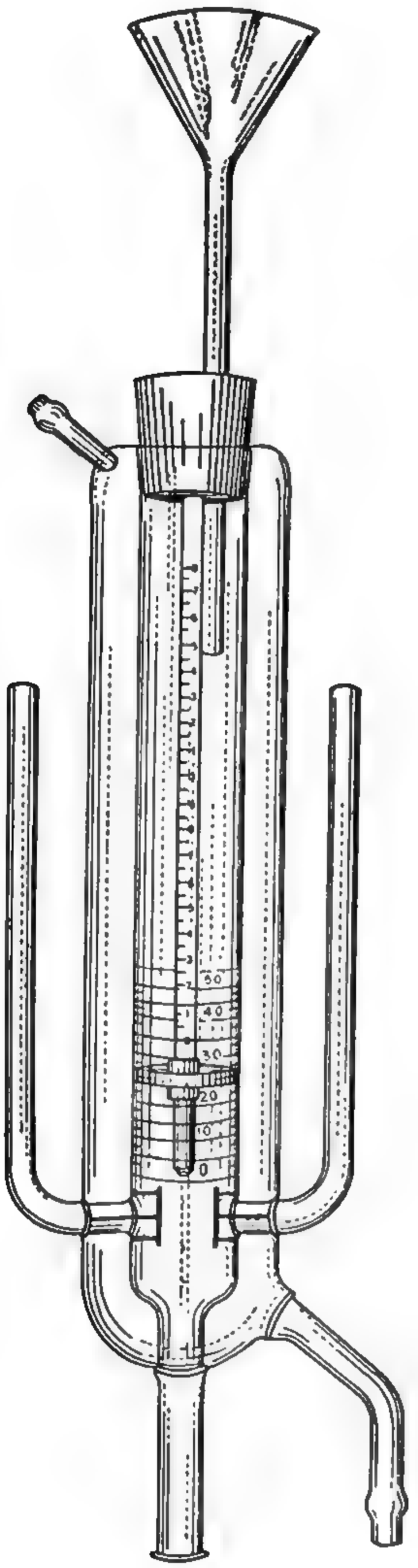


FIG. 234. Lange conductivity cell.

Conductivity Cell. For routine purposes the conductivity cell in Fig. 233 is preferably replaced by the Lange[68] type of cell, depicted in Fig. 234. It consists of an inner glass cylinder, 2.5 cm. in diameter and 15 cm. long, narrowing down at the bottom to a tube of 1-cm. diameter. A piece of rubber tubing with a pinchcock is attached to the lower end, to empty the cell. The electrodes are rectangular pieces of sheet platinum, 2 by 3 cm. in size, placed vertically, and firmly fastened to the walls of the cell by thin glass rods. A platinum wire affixed to each electrode passes through the wall of the cell into the side arm, which is filled with mercury, to connect the cell with the measuring equipment. The cell is closed at the top by a rubber stopper through which a funnel is inserted, to introduce the solution. The temperature is measured with a thermometer the top of which is fastened in the rubber stopper, and the bulb of which is placed directly above the electrodes. It is divided into tenths of a degree Centigrade.

[68] *Z. Ver. deut. Zucker-Ind.*, 60, 359 (1910).

The cell has a millimeter scale engraved on it so that a constant volume of solution may always be used. This is necessary because the current passes not only directly between the electrodes, but partly also through the solution above and below them. The entire cell is surrounded by a fused-on glass jacket, through which water of the desired temperature may be circulated.

There are many other types of conductivity cell, the design of which depends on the purpose for which they are to be used. For routine work of moderate precision the so-called dipping cell is often recommended. It consists of a pair of electrodes mounted inside a wide glass tube which is open at the lower end. The liquid to be measured is placed in a beaker or jar, and the cell is dipped into it. This type of cell is very simple and inexpensive, but the flow-through type of cell, described above, is preferable because of its rigid construction and easy temperature control.

Cell Constant. When a column of a conductor, 1 cm. long, and of 1-cm.^{2} cross section, offers a resistance of 1 ohm, it is said to have a specific resistance of 1. Hence

$$R = \frac{r \times l}{f}$$

where R is the resistance, l the length in centimeters, f the cross section in square centimeters, and r the specific resistance. The reciprocal of r is the specific conductance, designated by the letter κ, and expressed in reciprocal ohms, or "mhos," per centimeter (cm./cm.^{2} = 1/cm.).

The specific conductance of a solution can thus be determined by measuring the resistance of a column of known dimensions. In practice this is difficult to accomplish, and the usual course is therefore to calibrate a given conductivity cell with a solution of known specific conductance. The so-called cell constant or cell capacity is the product of the known specific conductance and the resistance, in ohms, of the standard solution. Since the resistance is measured in ohms, and the specific conductance in mhos per centimeter, the dimension of the product is 1/cm. The cell constant is therefore expressed in reciprocal centimeters. Its value for the cell described above is approximately 0.15 cm.$^{-1}$ When the cell constant has once been determined, the specific conductance of an unknown solution is found by dividing the cell constant by the resistance of the solution.

Before any measurements are made, the cell and the electrodes are thoroughly cleaned with chromic acid mixture, and then washed repeatedly with distilled water. In order to coat the electrodes with platinum black, a solution of 3 g. platinum chloride and 25 mg. of

lead acetate in 100 ml. of water is poured into the cell, and the latter is connected with a battery of four dry cells. The poles are reversed every 15 seconds for a period of 10 minutes. An even coating is thus deposited on the electrodes. The platinum chloride solution is drained off and kept in a bottle for future use. The cell is thoroughly washed with water and should always be kept filled with water when not in use.

An accurately tenth-normal solution of potassium chloride is prepared by dissolving 1.4911 g. of the purest salt (LaMotte), dried at 110° C., in a total volume of 200 ml. Twenty and 40 ml. of this solution are diluted at 20° C. to 200 ml., and the resistance of the 0.02 *N* and 0.01 *N* solutions thus obtained is determined, in the particular cell used, e.g, the Lange type of cell.

The cell is rinsed out twice with the 0.01 *N* solution, and then filled to a point well above the electrodes. The volume is finally adjusted to a definite mark, say 40 mm., on the scale engraved upon the cell. The extension coils are placed in the circuit by making connections with the outer binding posts on the slide-wire box. The slide wire is set at 500, and the dials on the resistance box are turned until a definite setting gives a minimum sound in the telephone receiver. Final adjustment is made by turning the slide-wire knob to the point where the sound practically disappears, preferably within the range of 475 and 525. At this moment the slide-wire setting, the number of ohms in the resistance box, and the temperature of the solution are noted. Two similar readings are taken, one with an extra ohm resistance, and another with 1 ohm less than the first one. This gives three independent determinations for one solution. The cell is drained, washed twice with the 0.02 *N* solution, filled with it, and three sets of readings are taken as before.

TABLE LXXIX

Temperature, °C.	Specific Conductance	
	0.02 *N* KCl	0.01 *N* KCl
15	0.002243	0.001147
16	0.002294	0.001173
17	0.002345	0.001199
18	0.002397	0.001225
19	0.002449	0.001251
20	0.002501	0.001278
21	0.002553	0.001305
22	0.002606	0.001332
23	0.002659	0.001359
24	0.002712	0.001386
25	0.002765	0.001413

From the data thus obtained the resistance is calculated by formula 2, p. 550, and the result is multiplied by the specific conductance of the potassium chloride solutions, shown for varying temperatures in the table of Kohlrausch and Holborn[69] (Table LXXIX).

Example. The following figures were obtained with the 0.01 *N* potassium chloride solution:

	Temperature, °C.	Slide-Wire Reading	Balancing Resistance
(1)	20.03	496	116 ohms
(2)	20.00	475	117 ohms
(3)	20.15	513	115 ohms

Taking the first setting, the resistance of the solution equals 116 × (4500 + 496)/(5500 − 496), = 115.81 ohms. By interpolating between the values given for 20 and 21° C. in the above table, the specific conductance of 0.01 *N* potassium chloride solution is found to be 0.0012788. The cell constant is therefore 115.81 × 0.0012788, = 0.14810 cm.$^{-1}$ Analogous calculations are made for the other two settings with the 0.01 *N* solution, and the three for the 0.02 *N* solution, and the six results are averaged.

The cell constant thus ascertained is correct only if water of zero conductance has been used for dissolving the potassium chloride. In practice even high-grade distilled water contains traces of electrolytes, and it is therefore necessary to determine the specific conductance of the water used, and to add the result to the specific conductance shown in Kohlrausch and Holborn's table.

The resistance of the distilled water is normally so great that it cannot be balanced at the center of the slide wire against the maximum of 9999 ohms of the resistance box. It is therefore necessary to connect with the inside binding posts on the slide-wire box, taking the extension coils out of the circuit. The resistance box is set at 9999 ohms, and the slide-wire knob is turned until the sound in the telephone receiver reaches a minimum. The specific conductance of the water is then calculated by formula 1 on p. 549. The water should be very close to 20° C., so that temperature corrections may be neglected.

Example. With distilled water in the cell, and with the resistance box set at 9999 ohms, the slide wire read 905. The resistance of the water is therefore (905 × 9999)/(1000 − 905), = 95,254 ohms. This is now divided into the approximate cell constant previously found, 0.14810, giving 0.0000016. The slight error in the cell constant does not affect this small figure.

The specific conductance of the water is added to that of the standard potassium chloride solution, and the sum is multiplied by the resistance of

[69] According to measurements by Jones and Prendergast, Kohlrausch's conductance values are about 0.2 per cent too high; *J. Am. Chem. Soc.*, **59**, 731 (1937).

the solution. The corrected cell constant is thus (0.0012788 + 0.0000016) × 115.81, = 0.14839.

Analogous calculations are again made for the other two settings with the 0.01 *N* solution, and the three with the 0.02 *N* solution, and the six results are averaged.

In routine work the calculations may be greatly simplified and expedited by the use of tables which give the logarithms of the values of $a/(1000 - a)$ and of $(4500 + a)/(5500 - a)$ for varying values of a.

Determination of the Specific Conductance. A solution containing 5 g. of a raw sugar in each 100 ml. of total volume is chosen as an example.[70] The cell is twice rinsed with this solution, and then filled to the 40-mm. mark, that is, the same as used in the determination of the cell constant. The resistance of the solution is balanced against a known resistance as previously described, with the extension coils in the circuit, and is calculated by formula 2 on p. 550. The resistance is divided into the cell constant previously determined, and the result is the specific conductance at the temperature of the measurement, but still uncorrected for the conductance of the water. If the readings were not made at exactly 20° C., a correction must be applied for the deviation. Lange found that the specific conductance of raw beet sugar solutions increases 2.187 per cent for each degree Centigrade increase, between 15° and 25°. For raw cane sugars Zerban and Sattler obtained the following formula, valid between 10° and 30° C.:

$$\kappa_t = \kappa_{20} \times [1 + 0.02234\ (t - 20) + 0.0000885\ (t - 20)^2]$$

This gives a change of 2.243 per cent per °C. between 19 and 21° C. If the measurements are made within this range, a rounded-off correction of 2.2 per cent is sufficiently exact, for both beet and cane sugars.

Finally, the specific conductance of the water used for dissolving the sugar must be deducted, if it is at all appreciable.

Example. A solution of 10 g. of a raw sugar in 200 ml. total volume gave a slide-wire reading of 507, when balanced against 720 ohms resistance, at 19.86° C. The resistance of the solution was therefore 720 (4500 + 507)/(5500 − 507), = 722.02 ohms. This figure, divided into the cell constant 0.14839, gives a specific conductance of 0.0002053. The temperature correction to be added is 0.14 × 2.2, or 0.308 per cent of this, = 0.0000006. The specific conductance, corrected for temperature, is therefore 0.0002059. By deducting from this figure the specific conductance of the water, 0.0000016, the final figure 0.0002043 is obtained for the corrected specific conductance of the solution.

[70] Zerban and Sattler, *Facts About Sugar*, **21**, 1158 (1926).

If the conductivity is used to calculate the ash content of sugar products, as is more fully explained in Chapter XVII, the conductance of the original solution must in certain cases be supplemented by conductance measurements in the presence of added acid or alkali.

Conductivity Determination in the Presence of Added Acid or Alkali. The reagents required for this purpose are 0.25 *N* hydrochloric acid, *N* phosphoric acid, and 0.25 *N* potassium hydroxide. The exact concentration of these most be controlled by measuring the specific conductance of 5 ml. of each diluted with 200 ml. of conductivity water, making 205 ml. in all. The specific conductance of the diluted hydrochloric acid must be 0.002370, that of the diluted phosphoric acid 0.001925, and that of the diluted potassium hydroxide 0.001422, all at 20° C., and corrected for the conductivity of the water employed.

Five milliliters of the 0.25 *N* hydrochloric acid, *N* phosphoric acid, or 0.25 *N* potassium hydroxide, respectively, is added to 200 ml. of the solution of the sugar product, which has been used for the determination of the original conductance. The mixture is thoroughly shaken, and its conductance is measured in the same manner as described previously. Corrections are applied for temperature, and for the conductance of the water.

The temperature corrections for the solutions to which alkali has been added are the same as given on p. 554 for the original solution without any addition. The corrections for the acidified solutions, however, are different. The following average correction formula is used:

$$\kappa_t = \kappa_{20}[1 + 0.01704\ (t - 20) + 0.000062\ (t - 20)^2]$$

The factor for $(t - 20)$ shows little variation but that for $(t - 20)^2$ fluctuates widely from one sugar to another. The conductance determinations in the presence of acid should therefore be made as closely as possible at 20° C.

After the conductivity determination in the acidified solution the cell is first washed with water, and then filled with 30 per cent methyl alcohol. This removes the acid which is strongly adsorbed by the platinum black. After the alcohol has been allowed to stand in the cell for a few minutes, it is drained off for future use, and the cell is washed and filled with water. In routine work time may be saved by first running all the samples without acid, and then all the acidified solutions.

With constant use, the electrodes gradually become coated with slimy material. To clean the cell, it is filled overnight with chromic acid mixture, and then thoroughly washed. After this treatment the cell constant must be redetermined.

THE DETERMINATION OF HYDROGEN-ION CONCENTRATION OR *p*H

By virtue of ionization, aqueous solutions exhibit a definite concentration of hydrogen ions. The concentration is, of course, dependent upon the nature of the dissolved substances and their degree of dissociation. It plays a very important role in many phases of chemical analysis and in the control of industrial processes; in fact, most life processes are also dependent upon some definite range of hydrogen-ion concentrations.

In sugar manufacture, hydrogen-ion concentration is correlated with the efficiency of clarification processes, of decolorization with carbons, and with the degree and rate of hydrolysis of sugars, starches, or other polysaccharides by acids or enzymes. The fermentation of sugars by microorganisms also proceeds within definite concentrations of hydrogen ions. Because of these facts the determination of hydrogen-ion concentration in the sugar factory as well as in control and research laboratories is now universally practiced. It is a measure of the effective acidity or alkalinity of the solution as compared with the gross acidity or alkalinity determined by ordinary titration.

Rather than use unwieldy fractions which accurately express the concentration of hydrogen ions, $[H^+]$, the more useful expression, *p*H, proposed by Sørensen,[71] has been very widely adopted. The mathematical relationship existing between *p*H and hydrogen-ion concentration is expressed by: $pH = \log_{10} \frac{1}{[H^+]}$. The actual concentrations of hydrogen ions encountered may range from 1 g. per liter for the most highly dissociated acid to $1/10^{14}$ (i.e., 10^{-14}) for the most highly dissociated base. On the *p*H scale the range is thus from 0 (strong acid) to 14 (strong base).

Buffer Action and Standards

If solutions of pure acids and bases were always encountered it would generally be possible to calculate the *p*H from their dissociation constants as most of these have long since been determined. However, in practice, solutions to be tested for their *p*H value are usually of rather complex nature. This may greatly alter the ionization of the acids and bases that might be present, and a *p*H value different from that which might be expected from the titration is obtained. The action of certain substances to repress the ionization of acids or bases or to resist a change in *p*H on the addition of acids or bases is called buffering action. All mixtures of weak acids and bases and their salts

[71] *Compt rend. trav. lab. Carlsberg,* **8**, 1, 396 (1909).

and of organic substances such as gelatin or casein exhibit pronounced buffering action.

The stability of a buffer system is dependent upon the total concentration and the ratio of the salt to the weak acid or base. Concentration effects are generally minor so that, as long as the ratio is kept constant, moderate dilution does not materially alter the *p*H value. This is of importance, for it is necessary at times to dilute solutions in order to measure their *p*H value, particularly those which possess color or turbidity and are measured by the colorimetric methods. However, caution should be exercised, and it is recommended that the specific effect of dilution be determined, in case of any doubt, in order to avoid undue errors.

There is a rather long list of buffer mixtures proposed by various investigators, the better known of which are perhaps those of Sørensen,[72] of Clark and Lubs,[73] and of McIlvaine.[74] The buffer standards of the last are the easiest to prepare and will suffice for a great many purposes. Their range is from 2.2 to 8.0 *p*H, and they can be prepared by mixing but two stock solutions, while the other two systems mentioned require from five to six stock solutions for the same *p*H range. These solutions are 0.2 *M* disodium phosphate ($Na_2HPO_4 \cdot 2H_2O$) and 0.1 *M* citric acid ($C_6H_8O_7 \cdot H_2O$). The phosphate with 2 moles of water of crystallization is prepared by exposing the ordinary crystals (with 12 moles of water) to the atmosphere for about 2 weeks. The air must be reasonably dry to achieve this transformation, and it is advisable to determine the actual water of crystallization prior to use to be certain of its composition. This is done by first drying the sample taken for analysis at 20 to 30 mm. pressure and 100° C. and then carefully igniting to constant weight. The loss in weight should be 25.28 ± 0.1 per cent. The citric acid should also be analyzed as it has a tendency to effloresce. Its water of crystallization should be determined by drying a sample at 20 to 30 mm. pressure and 70° C. The loss in weight should amount to 8.58 ± 0.1 per cent.

In Table LXXX are compiled the amounts of the phosphate and citric acid stock solutions (to make a total of 20 ml.) to be mixed together to yield solutions of definite *p*H value. For accurate work it is always advisable to check standard solutions for their *p*H value with an electrometric method after their preparation, as slight variations from those recorded occasionally creep in.

Conversely, there are many occasions when it is desirable to

[72] *Ergeb. Physiol.*, 12, 393 (1912).
[73] *J. Biol. Chem.*, 25, 479 (1916).
[74] *J. Biol. Chem.*, 49, 183 (1921).

check electrometric methods with a standard buffer. Several of these have been proposed, but potassium hydrogen phthalate has probably found greatest favor because of its ease of preparation and constancy of *p*H value. This standard buffer is prepared by dissolving 1.02 g. of pure potassium hydrogen phthalate crystals in 100 ml. of distilled water (1/20 mole solution). It has a constant *p*H value of 3.97 between 18° and 40° C.

TABLE LXXX

COMPOSITION OF MCILVAINE'S BUFFER STANDARDS

*p*H	0.2 *M* $Na_2HPO_4 \cdot 2H_2O$	0.1 *M* Citric Acid	*p*H	0.2 *M* $Na_2HPO_4 \cdot 2H_2O$	0.1 *M* Citric Acid
	ml	ml		ml	ml
2.2	0.40	19.60	5.2	10.72	9.28
2.4	1.24	18.76	5.4	11.15	8.85
2.6	2.18	17.82	5.6	11.60	8.40
2.8	3.17	16.83	5.8	12.09	7.91
3.0	4.11	15.89	6.0	12.63	7.37
3.2	4.94	15.06	6.2	13.22	6.78
3.4	5.70	14.30	6.4	13.85	6.15
3.6	6.44	13.56	6.6	14.55	5.45
3.8	7.10	12.90	6.8	15.45	4.55
4.0	7.71	12.29	7.0	16.47	3.53
4.2	8.28	11.72	7.2	17.39	2.61
4.4	8.82	11.18	7.4	18.17	1.83
4.6	9.35	10.65	7.6	18.73	1.27
4.8	9.86	10.14	7.8	19.15	0.85
5.0	10.30	9.70	8.0	19.45	0.55

COLORIMETRIC *p*H METHODS[75]

These methods involve the use of indicator dyes whose change in color is a function of the *p*H of the solution to which the dye has been added in definite proportions. As the perceptible color change of each of these dyes occurs only within definite and limited *p*H ranges, it is necessary to employ a number of dyes, individually, if the complete *p*H scale is to be covered. The indicator dyes which have become standardized for this work have been selected because of their trustworthiness. A list of these indicators is given in Table LXXXI with other pertinent information.

These indicators are available in crystalline form, or as 1 per cent and as proper working strength solutions. In preparing the indicator

[75] Colorimetric methods are fully described in such treatises as "The Determination of Hydrogen Ions" by W. Mansfield Clark (Williams and Wilkins Co., Baltimore, 2nd ed., 1928) and in trade publications as "The A B C of Hydrogen Ion Control" by the LaMotte Chemical Products Co., Baltimore, Md., and many others.

solutions from the crystalline form they are dissolved in the theoretical amount of sodium hydroxide solution to yield a *p*H as close as possible to the midpoint of their respective ranges, and are then made up to the proper strength as indicated in Table LXXXI. It is important that care be exercised to neutralize the indicators properly in preparing their solutions, particularly if they are to be used for determining the *p*H of slightly buffered solutions. Indicator solutions should be stored only in highly resistant glassware, sealed with glass or rubber stoppers, and in the dark.

TABLE LXXXI

INDICATORS FOR COLORIMETRIC *p*H METHODS

Indicator	Molecular Weight	Amt. *N*/20 NaOH 0.1 g. dye†	Concentration Employed	*p*H Range	Color Change
		ml.	per cent		
Acid cresol red	382	...	0.02	0.2 – 1.8	Red to yellow
Acid meta cresol purple	382	...	0.04	1.2 – 2.8	Red to yellow
Benzol yellow*	...	...	....	2.4 – 4.0	Red to yellow
Bromphenol blue	670	3.2	0.04	3.0 – 4.6	Yellow to blue
Bromcresol green	698	4.0	0.04	3.8 – 5.4	Yellow to blue
Methyl red	269	7.4	0.02	4.4 – 6.0	Red to yellow
Chlorphenol red	423	6.3	0.04	5.2 – 6.8	Yellow to red
Bromcresol purple	540	4.9	0.04	5.2 – 6.8	Yellow to purple
Bromthymol blue	624	4.8	0.04	6.0 – 7.6	Yellow to blue
Phenol red	354	7.7	0.02	6.8 – 8.4	Yellow to red
Cresol red	382	7.1	0.02	7.2 – 8.8	Yellow to red
Meta cresol purple	382	7.8	0.04	7.6 – 9.2	Yellow to purple
Thymol blue	466	6.5	0.04	8.0 – 9.6	Yellow to blue
Phthalein red*	...	...	....	8.6 –10.2	Yellow to red
Tolyl red*	...	...	....	10.0–11.6	Red to yellow
Parazo orange*	...	...	....	11.0–12.6	Yellow to orange
Acyl blue*	...	...	....	12.0–13.6	Red to blue

* These indicators available only in solution form.
† Personal communication from Dr. F. R. McCrumb, W. A. Taylor & Co., Inc., Baltimore, Md.

Approximate Colorimetric Methods. In this group are the simplified colorimetric methods which may be entirely suitable for many purposes but lack the refinements necessary for more accurate measurements. As will be noted from their description, the principal faults lie in the fact that it is extremely difficult to match the tints of solutions with printed charts even if they could be reproduced precisely and, in some cases, in the inability to compensate for the color or turbidity of the test solution.

Test papers serve for rough measurements, but here care must be taken in interpreting the color produced because of the possible selective adsorption of constituents from the solution by the paper. In

making a test, a strip of the test paper containing the indicator in whose range the *p*H of the solution falls is dipped in the solution to be tested for a sufficient length of time to insure equilibrium. The *p*H is estimated from the color produced, by experience or by comparison with printed charts.

Considerably more reliable results than are generally obtainable with test papers can be achieved with the spot-plate methods such as those developed in Hawaii[76] and Java,[77] and by Tödt[78] in Germany, for sugar-factory use. Briefly, these methods consist of adding definite amounts of the proper indicator and test solution together in the depression of a white porcelain test plate and comparing the resulting color with charts tinted to denote *p*H values in steps of 0.2 *p*H. The color which matches the solution being tested gives its *p*H value. The accuracy of such methods under ideal conditions is about 0.2 *p*H; for colored and turbid solutions this accuracy is apt to be reduced.

A rather unique device having about the same accuracy as the spot-plate methods is the Wulff *p*H tester.[79] The color standards consist of small celluloid strips impregnated with an indicator, the tints of which correspond to definite *p*H values in steps of 0.2 *p*H. Sets covering the range of 1.4 to 12.6 *p*H are available. In making a test, another celluloid strip containing the proper indicator is placed for 1 minute in the dish containing the solution or mixture to be tested. The strip is then removed, blotted to remove excess solution, and placed in a specially designed sliding holder where a comparison with the standards is quickly made. The *p*H is read off from the standard which most closely matches the test strip. It is claimed that this method is applicable to colored and turbid solutions provided that selective adsorption of the coloring matter contained in the test solution does not occur. Obviously if this occurred the final tint of the test strip could not be accurately matched with the standards.

Precise Colorimetric Methods.[80] In these colorimetric methods the proper indicator is first selected by trial. This can be done by employing one of the approximate methods just described or, preferably, by adding 0.5 ml. of the various indicator solutions of the proper

[76] "Chemical Control for Cane Sugar Factories," Assoc. of Haw. Sug. Tech., p. 65, 1931.

[77] "Handleiding voor Colorimetrische Zuurgradbepalingen," Java Sugar Expt. Sta., 1930.

[78] *Deut. Zuckerind.*, 60, 923 (1935).

[79] Pfaltz & Bauer, Inc., New York (agents).

[80] By precise is meant that the methods to be described have the greatest accuracy which it is possible to obtain with colorimetric methods. However, they generally lack the precision possible with electrometric methods.

strength to different 5- or 10-ml. portions of the solution to be tested, in agreement with the proportion prescribed by the manufacturers of the color standards. It will be observed that the color produced will be approximately the same as that obtained by the addition of pure alkali or acid to the indicators, with the exception of one or two; in other words, the *p*H will be beyond the limits of all but one or two indicators with overlapping ranges which are thus the ones to be used in making the *p*H determination of the solution in question.

This is done by adding to a specific quantity (5 or 10 ml.) of the solution to be tested 0.5 ml. of the indicator selected as just outlined, in a test tube having the same dimensions as the tubes containing the color standards employed. The contents of the tube are mixed, contact with the hands being avoided, and the resulting color is matched with the color standards. Matching is generally accomplished with the aid of some form of block comparator. In its simplest form, it consists of a block of wood or plastic, or of a metal rack, with two rows of three vertical holes each, to accommodate the test tubes, and ampules containing the color standards. Observation windows are placed in line, from the front to the back of the block, at right angles to each pair of vertical holes. The solution being tested and containing the required proportion of indicator is placed in the rear central hole with a test tube of water in the hole immediately in front. In each of the two remaining rear holes is placed a test tube containing a portion of the same test solution without any indicator. This is done to compensate the standard for any color or turbidity possessed by the solution being tested. For solutions which are too turbid or highly colored, moderate dilution can ordinarily be resorted to without serious error, but it is advisable to check with some suitable electrometric method in case of any doubt. In front of the two outside tubes are placed adjacent color standards in sequence until a color match is obtained by observation through the tubes. If a match is obtained by a standard at one end of a set its value is not accepted as the *p*H of the solution being tested since these colors are so close to the full color of the indicator (for acid or alkali) that the operator can be misled. The test should be repeated using the indicator whose *p*H range overlaps. A good source of daylight, real or artificial, is required to make accurate color comparisons. By this simple means *p*H determinations with an accuracy of about 0.1 *p*H can generally be obtained even with solutions containing some color and turbidity.

The above directions suffice for measuring the *p*H of the usually buffered solutions, but with slightly buffered solutions special technique is required because equilibrium can easily be upset by adsorption of

carbon dioxide or other vapors from the air and by using glassware not scrupulously clean. All glassware employed in handling slightly buffered solutions must be thoroughly cleaned and rinsed out with neutral water just prior to its use; also, the solutions must never be allowed to come in contact with the skin or even fingerprints. These points cannot be overemphasized.

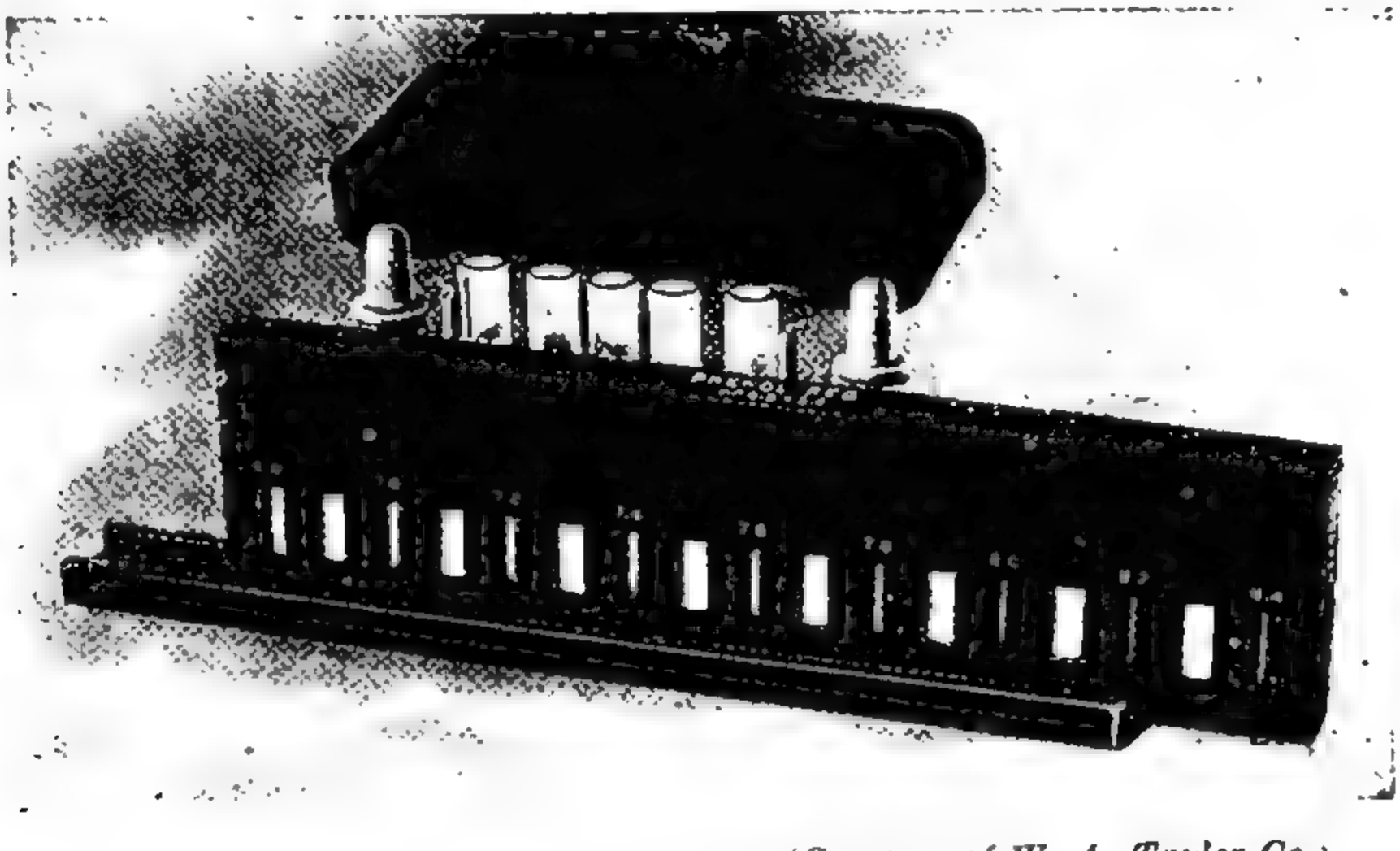
Ordinary distilled water, being in approximate equilibrium with the carbon dioxide of the air, shows a *p*H of about 5.5. Neutral water, with a *p*H of 7.0, can be obtained by redistilling a good grade of distilled water in which is dissolved barium hydroxide, using resistant glassware throughout. Even with protection against readsorption of carbon dioxide, the distillate generally has to be boiled to expel the last traces. This is done by boiling away from one-quarter to one-third of the redistilled water in a narrow-mouthed flask. The remaining portion is transferred, while still boiling hot, to a smaller container which can be completely filled. It is then cooled rapidly under a stream of cold water. Water prepared in this manner should consistently show a *p*H of 7.0 $\pm$ 0.05, using neutral bromthymol blue indicator solution.

If neutral water is to be used in preparing substances such as refined sugars, for determination of their *p*H value, it is necessary to exercise particular care to avoid contamination. In making the determination, the indicator solution is added to the test tube, recently rinsed with neutral water, before the solution to be tested. The latter is most conveniently added by means of a pipette, the tip of which discharges the solution at the surface of the liquid. In this manner the solution is not unduly exposed to the air and a good mixing of the indicator and solution results. Final mixing is obtained by giving the tube a rotating motion in a more or less vertical plane and not by stoppering and inverting. Comparisons with the standards are made quickly in the usual manner.

There are on the market a number of instruments based on the principle of the block comparator. In the Taylor Slide Comparator, Fig. 235,[81] each slide contains a complete set of standards for a given indicator in steps of 0.2 *p*H. The LaMotte Roulette Comparator is made circular in form and contains three sets of color standards, in steps of 0.2 *p*H; it is illuminated by an incandescent lamp with a filter

[81] Operation of Taylor Slide Comparator. "After removing top of base, three of the test tubes are placed in the holes back of the slots in the base and filled to the mark (5 ml.) with the sample to be tested. To the central tube 0.5 ml. of the indicator solution is added, by means of the pipette and nipple, and the contents thoroughly mixed. The color standard slide is then placed on the base and moved in front of the test samples until a color match is obtained. The *p*H is read directly from the value on the slide."

of "Dalite" glass. In both these apparatus the color standards in ampules alternate with tubes of water so as to meet the conditions described for the simple block comparator, when making a test. The Hellige Comparator is made of metal. It is similar in principle to a block comparator, but instead of standard solutions it has a circular holder with transparent disks tinted to match indicators in steps of 0.2 *p*H. The determination is made by observation through the



(Courtesy of W. A. Taylor Co.)

FIG. 235. Taylor slide comparator.

"block" with the tubes in place while the standards holder is rotated until a color standard (compensated in the usual manner) matches the color of the solution containing the proper proportion of indicator. The holders for the various indicator standards are interchangeable in the comparator so that the instrument serves the complete range from 0.2 to 13.6 *p*H.

Errors in Colorimetric Methods. There are certain errors which may arise in the determination of *p*H by colorimetric methods. These may be classified as salt effects, protein effects, influence of temperature, and certain specific effects due to inherent characteristics of some of the indicators.

As the color produced by an indicator in a buffer solution, which generally constitutes the standard, is influenced to a certain extent by the concentration of the salts present, it is obvious that in testing unknown solutions and comparing them with the standards an error might result because of a differential in salt concentration. If the concentration of salt in the solution being tested is lower than in the standard buffer the tendency is for the colorimetric method to indicate too low

results, and vice versa. These errors differ somewhat with the various indicators but may amount to as much as 0.3 *p*H for solutions whose salt concentrations range from 1/10 or 1/20 to 10 or 20 times that of the buffer standard. It would be extremely difficult in practice to arrive at any proper correction without checking the colorimetric with an electrometric method. Fortunately, in a majority of cases the errors are slight and have little significance as comparative values are of more importance than absolute ones.

Proteins in solutions tested for their *p*H value cause an error in colorimetric methods. The degree is influenced by a number of factors, such as the type of protein, its concentration, the indicator employed, and the *p*H of the solution. If such errors are encountered it is best to resort to a suitable electrometric method rather than attempt to apply a correction, since the degree of the error is not predictable unless tests are made under comparable conditions.

As temperature influences the ionization equilibrium of salt solutions, an effect of temperature on the *p*H value of solutions can be expected. Color standards are usually prepared for use at normal room temperatures (20° to 30° C.); hence *p*H determinations by the colorimetric methods should be made within this temperature range. If this is not possible, comparative measurements may be made without serious error provided that the tests are conducted at the same temperature.

Among the specific effects should be mentioned, particularly, the characteristic of certain indicators such as bromphenol blue and bromcresol purple to show a tint which varies with the concentration of dye and depth of layer. This is termed "dichromatism." This effect is noticeably exhibited in testing turbid solutions when it may be found impossible to obtain a match with the standards even though compensated in the usual manner. In such cases it is usually possible to substitute another dye, or else an electrometric method must be used.

Electrometric *p*H Methods[82]

Electrometric methods are based upon the principle of measuring the electromotive force (E.M.F.) generated, as a function of the hydrogen-ion concentration (and temperature) between electrodes of various types immersed in a solution to be tested and in a solution of known and definite characteristics joined thereto by a liquid junction (salt bridge).

[82] Electrometric methods are described fully in "The Determination of Hydrogen Ions" by W. Mansfield Clark (Williams and Wilkins Co., Baltimore, 2nd ed., 1928) and in trade publications by Leeds & Northrup Co., Philadelphia, Pa., and others.

The standard and classical electrometric method employs the hydrogen electrode. Because of the many difficulties involved in its use, the hydrogen electrode has found only limited application in sugar investigations. The quinhydrone electrode described by Biilmann,[83] and found applicable by Dawson[84] to the determination of the *p*H of many sugar-house products, was quickly adopted in investigational work relating to clarification, decolorization, and other problems of sugar manufacture, because of its simplicity and accuracy (within limits). The glass electrode introduced later has been quickly gaining favor because of its still greater simplicity and range over the quinhydrone electrode. Where ruggedness is necessary, as for the automatic control of factory processes, the antimony electrode is being widely used in the sugar industry. Suitable glass electrodes[85] have become available for similar services, but sufficient time has not elapsed to indicate whether they can replace the antimony electrode for sugar-factory use. Pertinent information concerning each of these types of electrodes and a description of devices for measuring the E.M.F. of the hydrogen-ion cells involved will be given in some detail.

Hydrogen Electrode. The hydrogen electrode consists of a platinum or gold foil carefully plated with platinum, palladium, or iridium, and immersed in the solution being tested which is saturated to equilibrium with purified hydrogen gas. The hydrogen is bubbled through the solution surrounding the electrode, or the solution is shaken in an atmosphere of hydrogen in a specially designed chamber. In order to measure the electric potential of the solution in which the hydrogen electrode is placed, it is brought in contact by liquid junction with another electrode or half-cell which may be a similar hydrogen electrode in a solution of known *p*H value, or it may be one of the other types of standard half-cells such as one of the calomel electrodes.

The calomel electrode is composed of mercury and calomel (mercurous chloride) in a water solution of potassium chloride. These materials are contained in a glass vessel of which many designs are available. Provision is made in some manner (by stopcock, plug, or sleeve) to protect the electrode from contamination by diffusion of the solution being tested for its *p*H value through the salt bridge, and to flush out with fresh potassium chloride solution. Electrical contact to the calomel cell is obtained through the mercury by means of a platinum wire sealed through the bottom of the calomel electrode vessel or in a tube inserted through the top opening of the vessel. The

[83] *Ann. chim.*, (9) **15**, 109 (1921); (9) **16**, 321 (1921).

[84] *Sugar*, **28**, 211, 262, 310, 369 (1926).

[85] Beckman Shielded Glass Electrode by National Technical Laboratories, Pasadena, Calif.

potential of a calomel electrode is dependent upon the concentration of the potassium chloride solution in contact with the calomel and mercury. One of three concentrations may be used, namely, 0.1 *N*, *N*, or saturated. The last is used most widely in practice because it is easily prepared; it has the same salt concentration as the salt bridge and hence eliminates diffusion difficulties; and it has a high conductivity which increases the sensitivity of the system.

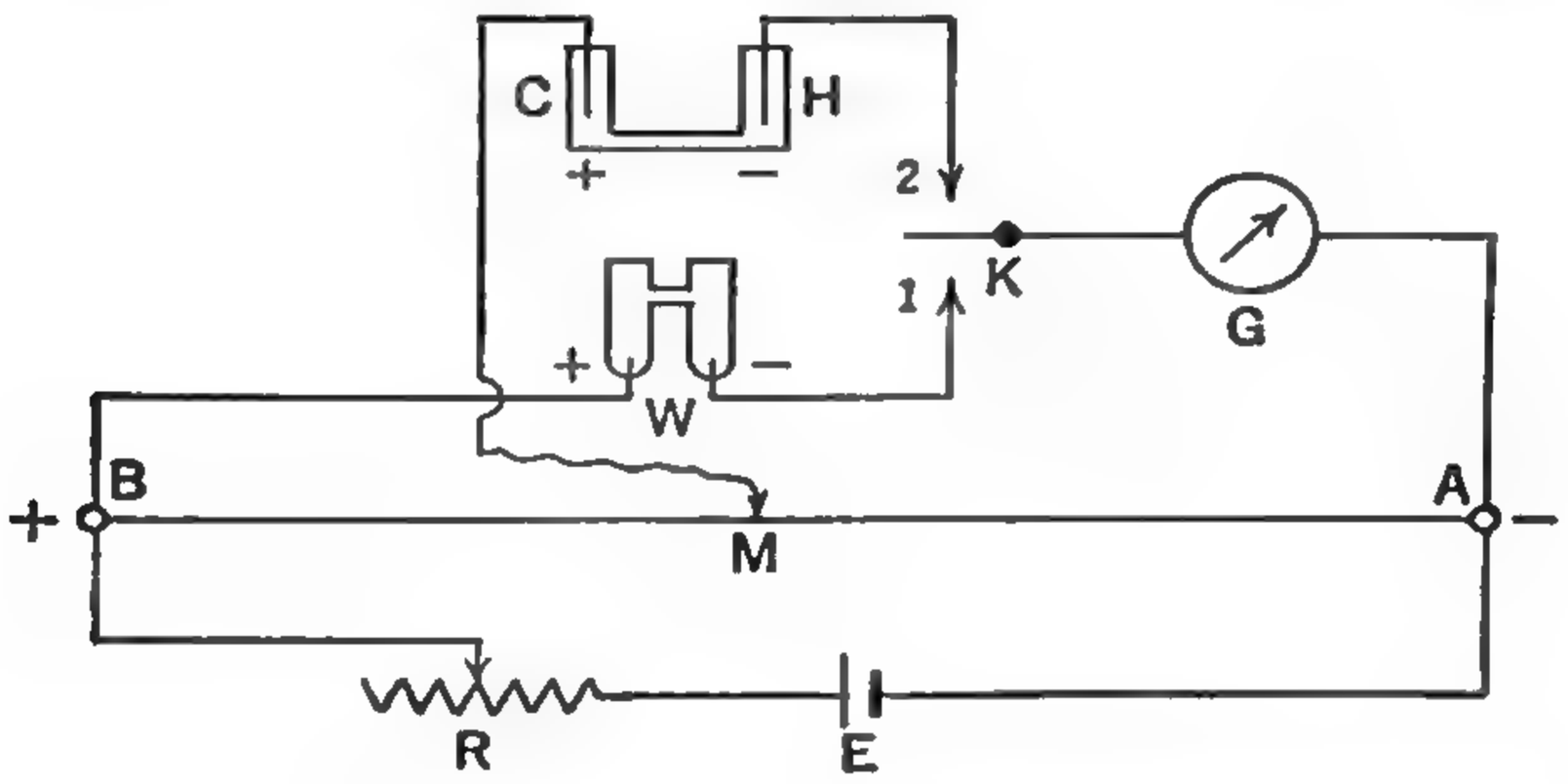
In preparing calomel electrodes, specially purified mercury and calomel should be used in order to obtain correct potentials. Chemically pure potassium chloride is suitable for preparing the salt solutions. Enough mercury to cover the platinum wire contact is first added to the electrode vessel, then sufficient calomel to insure an excess at all times, and finally the potassium chloride solution of the desired strength (that is, 0.1 *N*, *N*, or saturated) is added. The potassium chloride solution may be saturated with calomel prior to its addition to the electrode vessel to aid it in coming quickly to equilibrium. When properly joined to the salt bridge which may be an integral part of the calomel electrode vessel, it is ready for use, in conjunction with the hydrogen or other types of electrodes designed for *p*H determinations.

The salt bridge is a saturated potassium chloride solution, with or without agar. Agar is sometimes used to form a gel and to reduce diffusion, because it has a high conductivity and it obviates the making of liquid junction potential corrections except when undertaking fundamental *p*H studies.

In making *p*H measurements of the highest precision with the hydrogen electrode it is necessary also to take into consideration the barometric pressure, as it is presumed that the pressure of the hydrogen gas at the surface of the hydrogen electrode is 1 standard atmosphere (760 mm. of mercury at 0° C.), whereas actually the pressure varies with the atmospheric pressure which in turn affects the potential of the electrode. Fortunately, this correction generally amounts to considerably less than 0.02 *p*H and can ordinarily be neglected.

The fundamental equation linking *p*H with potential measurements with the use of two hydrogen electrodes, is expressed by: $\log C_n/C = V/0.0001983\,T$, where C_n is the concentration of hydrogen ions in the known solution, C that in the unknown solution, V the potential in international volts, and T the temperature in degrees absolute (degrees Centigrade plus 273.1). If the known solution contains the normal concentration of hydrogen ions, C_n equals 1, and C_n/C becomes $1/C$. The log of this fraction by definition equals *p*H. Hence $pH = V/0.0001983\,T$. At 25° C., at which *p*H determinations are usually made, $pH = V/0.0591$. The potential of the theoretical normal hydro-

gen electrode is assumed to be zero at all temperatures; hence, when a reference electrode other than the normal hydrogen electrode is introduced, the measured voltage V must be corrected for the difference between the reference electrode and the normal hydrogen electrode v. The equation is thus generally written: $p\text{H} = \frac{V - v}{0.0001983\ T}$. For the saturated potassium chloride-calomel electrode, which is the type most widely used, the correction $v = 0.2650 - 0.00077\ t$, where t is temperature in degrees Centigrade, between 18° and 40°. Substituting this correction, the equation becomes $p\text{H} = \frac{V - (0.2650 - 0.00077\ t)}{0.0001983\ T}$ and is applicable when both electrodes are at the same temperature.



(Courtesy of Mr. R. T. Balch.)

FIG. 236. Diagram showing principle of potentiometer circuit.

As electrometric pH determinations are based on the measurement of voltage, suitable equipment with which to measure this voltage is as essential as the hydrogen or other electrode system. It is imperative that this equipment be capable of measuring the difference in potential between the two electrodes without drawing any appreciable current which would cause polarization of the electrodes and erroneous results. A potentiometer is well suited for this service and is the type of instrument generally used.

In Fig. 236 is shown a diagrammatic sketch of a simple potentiometer circuit which will explain the principles involved. The working battery E supplies a constant voltage to a wire conductor AB of uniform resistance stretched along a scale graduated in uniform divisions. This scale may be graduated in terms of volts or directly in pH units when calibrated for the use of certain types of electrodes under definite conditions. The constant voltage in AB is always made equal to that furnished by a standard cell W, prior to every test, by pressing key K to

position 1 and adjusting the rheostat *R* until no deflection of the galvanometer is obtained. When the key *K* is closed in position 2 the hydrogen-ion cell *C–H* is connected to the potentiometer circuit. Since the calomel electrode *C* is always more positive than the hydrogen electrode it is connected at *B*, the positive side of the circuit, and the hydrogen electrode *H* is connected to the negative side. The circuits connected in this manner oppose one another, and the potential difference between the calomel and the hydrogen electrodes can be made equal to the potential drop between some two points, say *A* and *M*, in the potentiometer circuit *AB*. This is accomplished by moving the sliding contact *M* along *AB* until no deflection of the galvanometer is observed. The reading obtained from the scale corresponding to the setting of the slide gives the voltage generated by the hydrogen-ion cell or the *p*H of the solution being tested.

Potentiometers based on the above principles are available in a variety of designs and sensitivities to meet any laboratory or factory use with the hydrogen, quinhydrone, or antimony electrodes. The glass electrode requires an instrument of much higher sensitivity because of its exceedingly high resistance, and for this service a type of potentiometer in which the voltage produced by the hydrogen-ion cell is amplified by means of an electronic tube has been developed. This will be described in a little more detail under the glass electrode.

Quinhydrone Electrode. The quinhydrone electrode consists simply of a plain gold or platinum electrode immersed in the solution to be tested, which is saturated with quinhydrone. Quinhydrone in solution dissociates into hydroquinone and quinone which ionizes. In a solution which contains hydrogen ions in the presence of constant and equal proportions of the dissociation products of quinhydrone, the potential is directly proportional to the *p*H. This condition exists in acid solutions saturated with quinhydrone provided that a small excess remains undissolved. In solutions more alkaline than approximately *p*H 8 the quinhydrone becomes more soluble and the hydroquinone dissociates and oxidizes. This causes the relationship between *p*H and voltage to deviate from a linear function and hence limits the use of the quinhydrone electrode for the measurement of the *p*H to solutions whose value is not more than from 8 to 9 *p*H.

To complete the hydrogen-ion cell, the quinhydrone electrode is connected by liquid junction to a reference electrode which may be another quinhydrone electrode in a buffer solution of known *p*H value or, as is more common, the reference electrode may be one of the calomel cells already described. Temperature influences the voltage in much the same manner as with the hydrogen electrode.

The relationship between a quinhydrone reference electrode potential and *p*H at the same temperature can be expressed by the equation:

$$pH = \frac{0.7177 - 0.00074\,t - V - v}{0.0001983\,T}$$

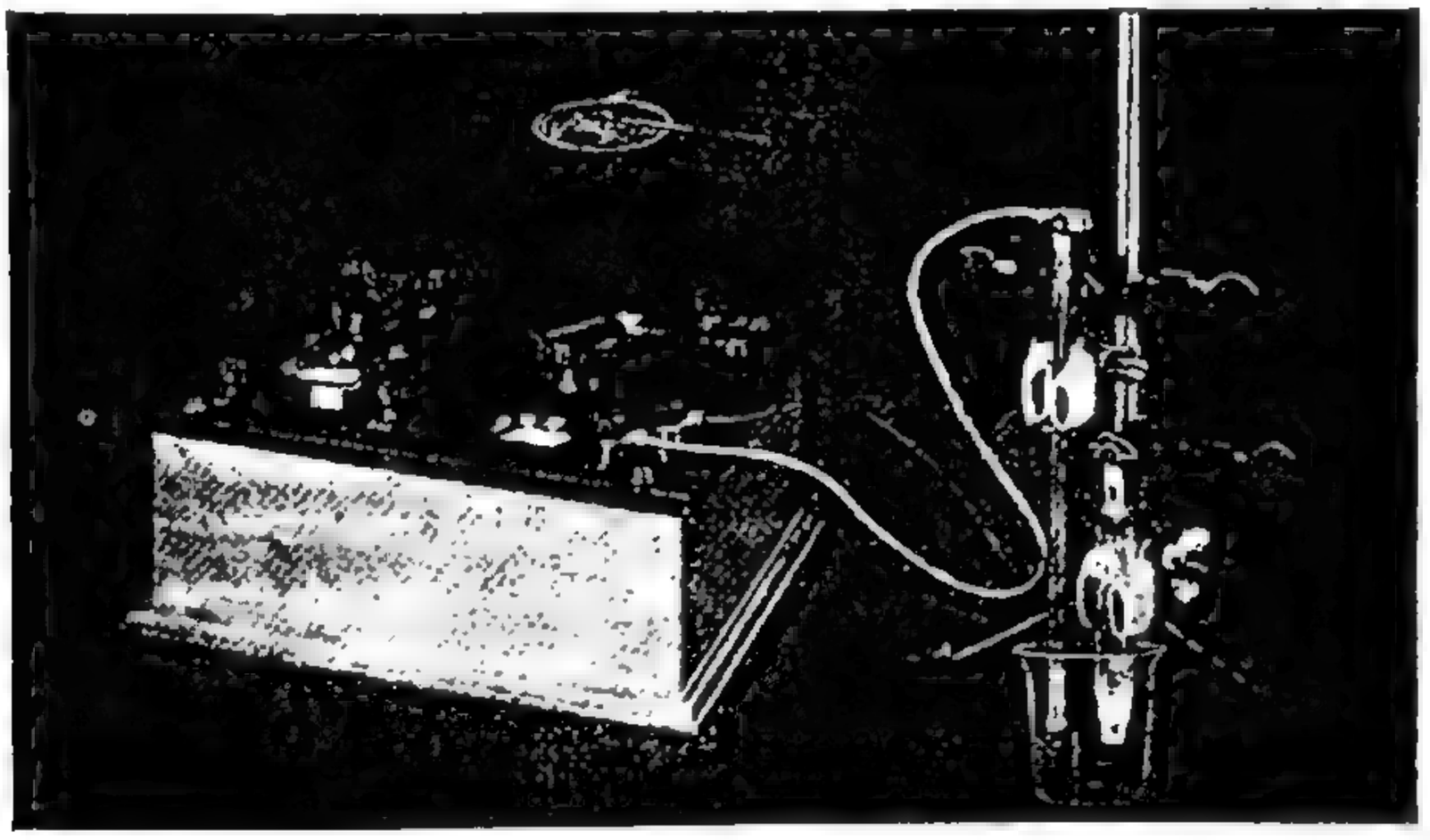
where t = degrees Centigrade, T = absolute temperature, V = observed potential, and v = potential of the reference electrode. If a saturated potassium chloride-calomel electrode is used the equation becomes:

$$p\text{H} = \frac{0.4527 + 0.00003\,t - V}{0.0001983\,T}$$

or at 25° C.,

$$p\text{H} = \frac{0.453 - V}{0.0591}$$

At a *p*H of 7.67 (25° C.) the potential of the quinhydrone equals that of the saturated potassium chloride-calomel cell, and V becomes zero.



(Courtesy of Leeds and Northrup Co.)

FIG. 237. Leeds and Northrup quinhydrone *p*H indicator.

At *p*H measurements more acid than 7.67 the calomel electrode is connected to the negative post of the potentiometer and the quinhydrone electrode to the positive. These connections must be reversed in measuring solutions more alkaline than 7.67 *p*H unless the potentiometer circuit is designed especially to care for this situation.

In Fig. 237 is shown a simple assembly for determining *p*H with the quinhydrone electrode. This equipment consists of a potentiometer for measuring the potential between the quinhydrone and saturated potassium chloride-calomel electrodes extending into the solution being tested, contained in a beaker. This particular potentiometer is gradu-

ated in terms of volts (—400 to +100) and *p*H (1 to 9). The latter scale is applicable only when the measurements are made at 25° C. The instrumental error is equivalent to ±0.04 *p*H and the electrode limit of error, ±0.01 *p*H; if greater precision is desired a potentiometer having a lower instrumental error and greater sensitivity should be employed.

To make a *p*H determination with this equipment, first a slight excess of quinhydrone crystals is added to the solution to be tested, which is then stirred for a moment to insure saturation; second, the platinum or gold and calomel electrodes are lowered into the test solution to a depth completely covering the metal electrode and the end of the salt bridge surrounding the calomel electrode; third, the voltage of the hydrogen-ion cell is measured with the potentiometer previously balanced in accordance with the manufacturer's instructions, the temperature being noted. The *p*H can be read from the scale if the determination is conducted at 25° C., or it can be calculated from the voltage reading by using the formula given above or by referring to appropriate charts or tables usually furnished with the equipment.

A drift of potential may be noted at times when a determination is made. This may be due to an insufficient excess of quinhydrone to meet the conditions for obtaining equilibrium between the products of dissociation, or there may be a slow reaction between the quinhydrone and certain substances in solution. Generally, the stability of the potential is an index of the reliability of the measurement, and the reading most reliable in cases of a slow drift is that observed just before the drift begins. Salt and protein errors are generally negligible.

As already indicated, the quinhydrone electrode does have some limitations, in that it is not suitable for determining the *p*H of solutions having a value much higher than 8; it is possible, however, to measure the *p*H up to about 9 without serious error if the solution is well buffered. It should be mentioned too that this electrode is not applicable to solutions containing oxidizing or reducing substances, such as sulfur dioxide, sulfites, chlorine, or other bleaching agents. When such solutions are encountered it is sometimes possible to use a colorimetric method, but more certain results are obtained with a glass electrode.

Glass Electrode. Determinations of *p*H have been greatly simplified by the use of glass electrodes and the necessary potential measuring devices of high sensitivity. Glass electrodes, as the name implies, are bulbs of thin-walled glass of special composition blown on the end of a glass tube, which is immersed in the solution to be tested. Inside this tube is an electrode of some type, such as a quinhydrone electrode in an acid solution. It is believed that an actual transfer of hydrogen ions

takes place through the bulb which makes it behave like a hydrogen electrode. As with the hydrogen and the quinhydrone electrodes, a reference electrode and salt bridge are used in conjunction with the glass electrode to complete the hydrogen-ion cell.

In many respects the glass electrode is considered ideal in that nothing has to be added to the solution which might alter its hydrogen-ion concentration, that the electrode cannot become poisoned, and that it can be used for measuring the *p*H of all kinds of materials including those which are semi-solid in consistency and those which contain active reducing or oxidizing substances. The range of application is normally from about 1 to 13 *p*H; however, errors may be introduced in alkaline solutions containing appreciable amounts of sodium salts. With frequent and proper calibration a limit of error of about ± 0.01 to 0.02 *p*H unit is attainable with the glass electrode.

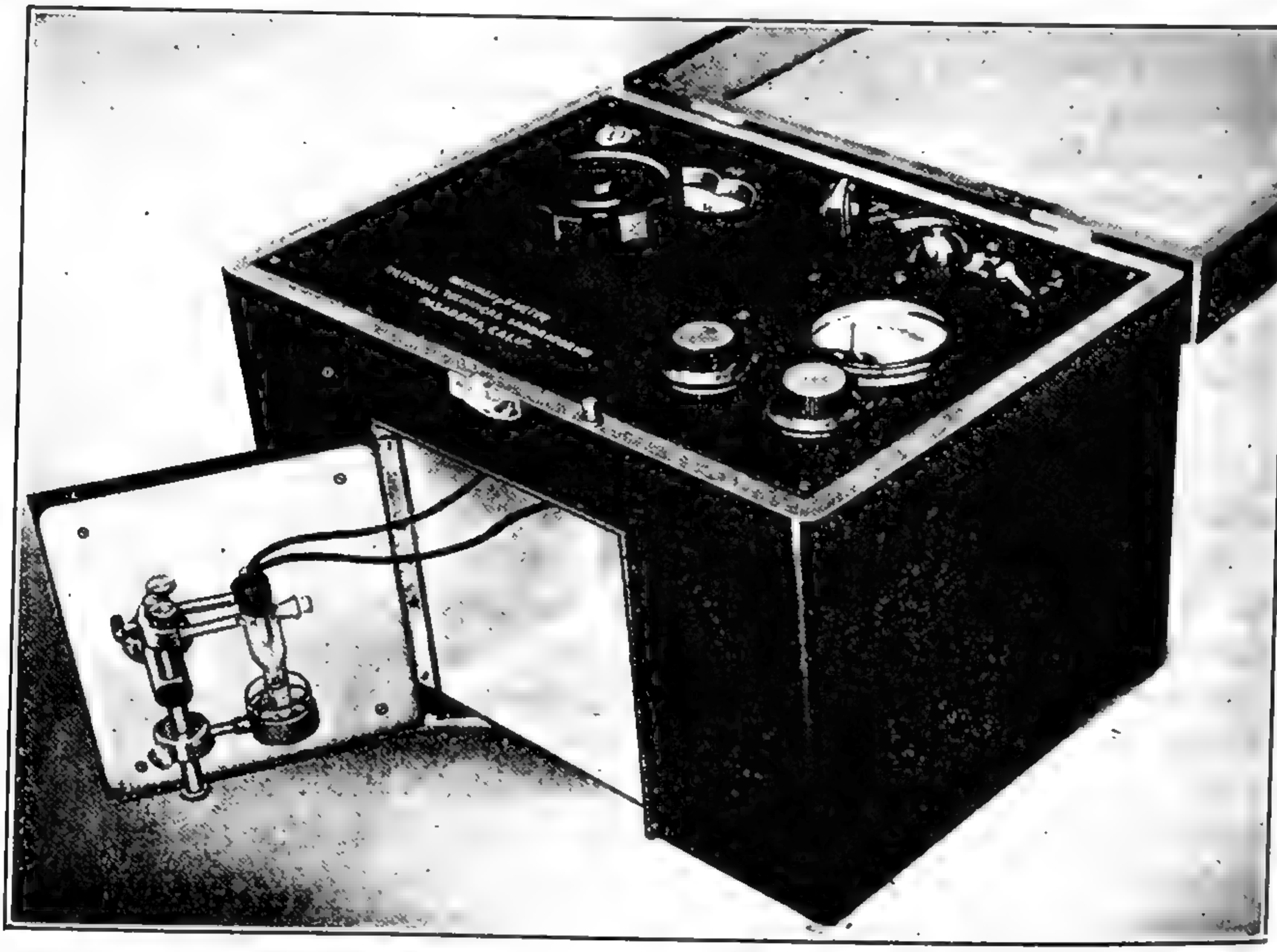
Several makes of glass electrode *p*H equipment[86] are on the market all of which operate on more or less similar principles. The main differences between them are structural details.

One of these, the Beckman laboratory model *p*H meter, is illustrated in Fig. 238. The electrode system of this equipment consists of a factory-sealed glass electrode and a saturated potassium chloride-calomel electrode. These electrodes are made in a variety of shapes and sizes; normally they are small and sturdy and require no maintenance except the usual rinsing off and resupplying the saturated potassium chloride solution constituting the salt bridge which surrounds the calomel electrode. They are mounted on a bracket on the door to a compartment in the front of the potentiometer box. The sample to be tested, of which only a few milliliters are required, is contained in a small cup. The cup is raised, after filling, to the correct position covering the tip of the glass electrode and of the salt bridge surrounding the calomel electrode. Convenient electrical connections are made to the potentiometer.

As with other types of potentiometers, the voltage developed at the electrodes of the hydrogen-ion cell is connected in series opposition with a variable voltage from a precision slide-wire potentiometer circuit. In this case, however, it is necessary to amplify the differential voltage on account of the extremely high resistance of the glass electrode. This is accomplished with an electronic tube of specific characteristics. The

[86] Beckman, manufactured by National Technical Laboratories, Pasadena, Calif.
Coleman, manufactured by Coleman Electric Co., Maywood, Ill.
Universal *p*H Indicator, manufactured by Leeds & Northrup Co., Philadelphia, Pa.
Hellige, manufactured by Hellige, Inc., Long Island City, N. Y.
Thwing, manufactured by Thwing-Albert Instrument Co., Philadelphia, Pa.

amplified voltage is used to operate a balance-indicating meter, and the potentiometer is adjusted until the differential voltage becomes zero. The *p*H (or voltage) is read from the calibrated potentiometer scale. In these types of instruments provision is made to compensate for the effect of temperature upon the voltage of the hydrogen-ion cell; the compensator is incorporated in the electrical circuit and can be set, in this instrument, to correct automatically for temperatures between 10° and 40° C. over the entire range of 0 to 13 *p*H (0 to 1300 millivolts). It is also necessary with electronic potentiometers to guard against electrical leakage and the picking up of stray currents which might be amplified, and thus give erroneous results. This is done by proper shielding. Prior to making an actual *p*H measurement with this equipment, it has to be adjusted to care for any changes which might have occurred in the battery voltage and for tube filament and glass electrode characteristics. This is accomplished by adjusting appropriate and convenient controls, a full description of which is furnished with the instrument.



(Courtesy of National Technical Laboratories.)

FIG. 238. Beckman laboratory *p*H meter with glass electrode.

Antimony Electrode. The first development of an electrode for continuous factory service was the tungsten electrode, and its application in the sugar industry has been described by Balch[87] and by Balch and Keane.[88] The tungsten electrode has since been replaced for such usage by an antimony electrode because of its greater trustworthiness.

The antimony electrode, consisting of a block of specially prepared antimony mounted with one end exposed to the air and the other dipping into the solution being tested, requires a flowing or agitated solution to give reproducible results. The relationship between *p*H and measured voltage from the hydrogen-ion cell varies with the nature and concentration of the solution being tested. Fortunately, however, it follows one of a series of definite curves which can be selected by trial. The antimony electrode is suitable for the normal range of about 4.0 to 11.5 *p*H with an accuracy of ± 0.2 *p*H, although the range and accuracy can be increased under special conditions.

For the automatic control[89] of juice clarification, in beet and cane sugar manufacture, the electrode assembly consists of an antimony and saturated potassium chloride-calomel electrode unit. These electrodes are mounted in a flow chamber, through which a continuous sample of juice passes, along with the resistance bulbs which automatically compensate the voltage measurements for temperature. The voltage generated by the hydrogen-ion cell is measured and recorded continuously in terms of *p*H by means of a recording potentiometer which, in turn, controls the addition of lime or of gas to the process through auxiliary feeding units at a desired *p*H value. The many installations which have been made during the past few years are indicative of the value of automatic *p*H control in the sugar industry.

Electrometric *p*H Measurement at High Temperature. It is frequently desired to know the *p*H of hot factory juices and sirups, in order to ascertain the danger of inversion, of the destruction of reducing sugars, etc. Since the *p*H varies with the temperature, the correct result cannot be obtained in such cases by cooling the solution to room temperature and then determining its *p*H. Spengler, Böttger, and Seeliger[90] have described special equipment by means of which *p*H measurements may be made at temperatures up to 100° C. with the hydrogen electrode, and up to 80° C. with the quinhydrone electrode.

[87] *Louisiana Planter*, 75, 347 (1925).

[88] *Ind. Eng. Chem.*, 20, 1148 (1928).

[89] This equipment is manufactured by Leeds & Northrup Co., Philadelphia, Pa., under the trade name of "Micromax Automatic *p*H Control."

[90] *Z. Ver. deut. Zucker-Ind.*, 88, 295 (1938).

MEASUREMENT OF COLOR OF SUGAR PRODUCTS

Color is defined by the physicist as the sensation due to the stimulus of the optic nerve. Strictly speaking, color itself cannot be measured, but only the stimulus evoking it. The fundamental method for accomplishing this is spectrophotometry; various empirical methods, synthetic in nature and founded on psychophysiological phenomena, are also in use in various industries. The sugar chemist is, as a rule, not primarily interested in *color* as defined by the physicist, but wants information on the quantity and nature of the *coloring matter* present in sugar products.

Colorimetry

In many cases the quantity of a colored substance in solution can be determined with a so-called colorimeter; this term is really a misnomer, because the instrument does not measure color in the physical or physiological sense, as might be implied from its name. Its use is based on the fact that, when two solutions of the same colored substance, but differing in concentration, absorb the same amount of light and therefore show the same brightness, the concentrations, c and c_1, respectively, are inversely proportional to the heights, h and h_1, respectively:

$$c : c_1 = h_1 : h$$

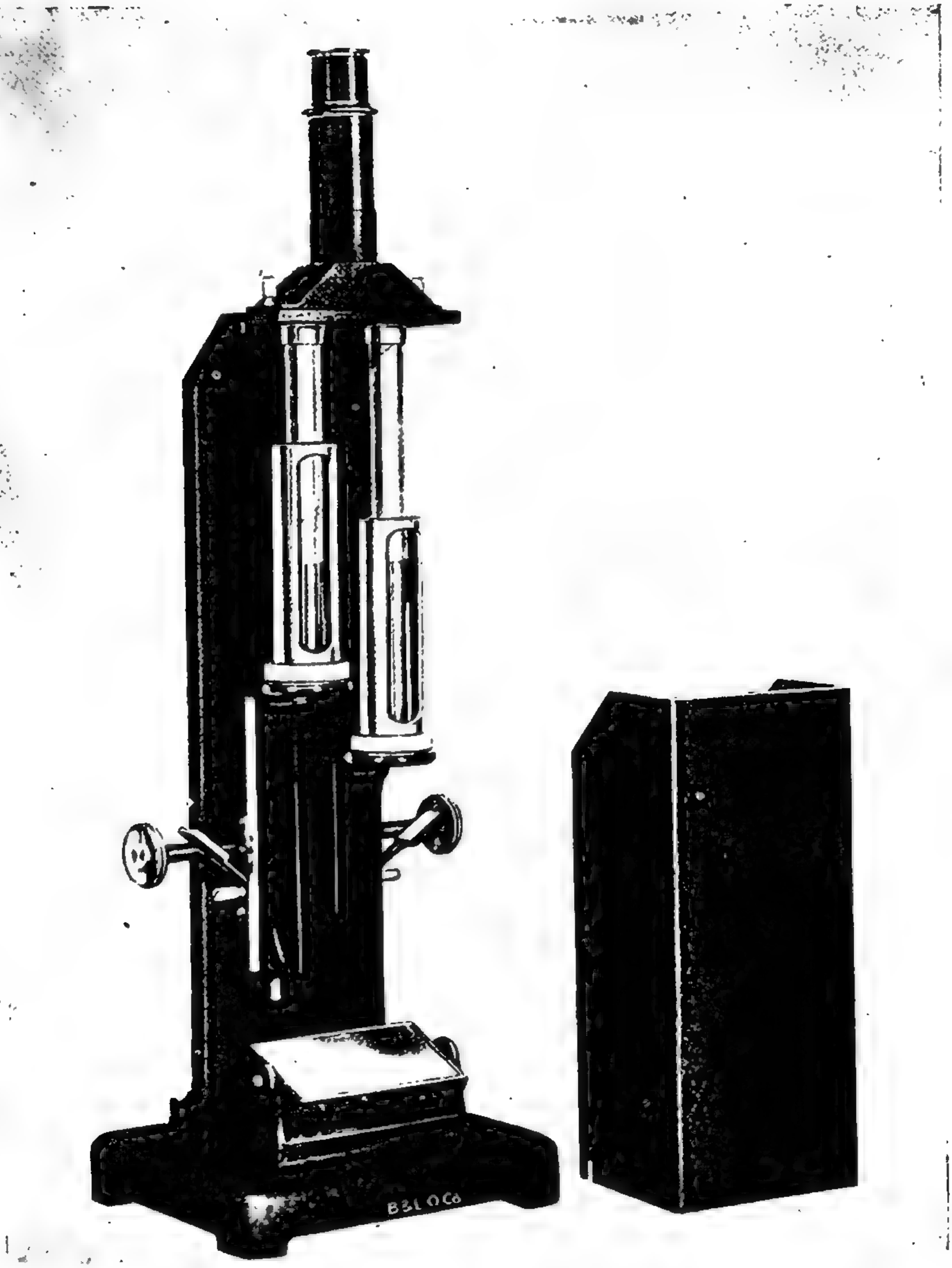
If the concentration of one solution is known, and the heights of the two solutions are adjusted to equal brightness, then the concentration of the other can be calculated from the above formula:

$$c_1 = \frac{c \times h}{h_1}$$

The colorimeter of Duboscq, one of the best known, is selected for description.

Duboscq Colorimeter.[91] This instrument is made in several sizes, with slight variations in constructional details. For general purposes maximum depths of 100 or 50 mm. are used; for biological work a simpler instrument, with 40-mm. depth, is made; and for micro work one with 20-mm. denpth. The 100-mm. instrument is shown in Fig. 239 and diagrammatically in Fig. 240. It consists of an upright case, with hinged or removable cover. The light is reflected into the instrument by a mirror M, which rotates around a horizontal axis and has two reflecting surfaces, one silvered, and the other of opal glass to furnish diffuse light. Above the mirror are two cups, C and C_1, which are

[91] Courtesy of Bausch and Lomb Optical Co.



(Courtesy of Bausch and Lomb Optical Co.)

FIG. 239. Duboscq colorimeter.

moved up and down by a rack and pinion. They are firmly connected with two scales on which the height of the liquid columns can be read from the eyepiece position by means of two mirrors set at an angle of 45°. The scales are divided into millimeters and are read with a vernier to 0.1 mm. The cups consist of glass cylinders fitted into nickeled casings which are threaded at the lower end. A metal screw cap forces a plane glass plate firmly against the cylinder end, which is finely ground to be water tight. The bottom of the cup is an optically inactive glass disk with perfectly plane parallel surfaces. A rubber washer between the disk and cup equalizes the pressure and provides for easy adjustment of a number of cups to perfect identity of 0 point. Two plungers of glass, T and T_1, hexagonal in form, and also with plane parallel lower ends, are attached to the frame of the instrument. The prism combination P and P_1 consists of a double rhomboid reflecting system with a biprism refracting system. It gives a practically invisible dividing line in the field F which exactly resembles the field of a polariscope and is observed by means of a microscope. Either daylight, artificial white light, or monochromatic light may be used according to requirement. Special colorimeter lamps are available.

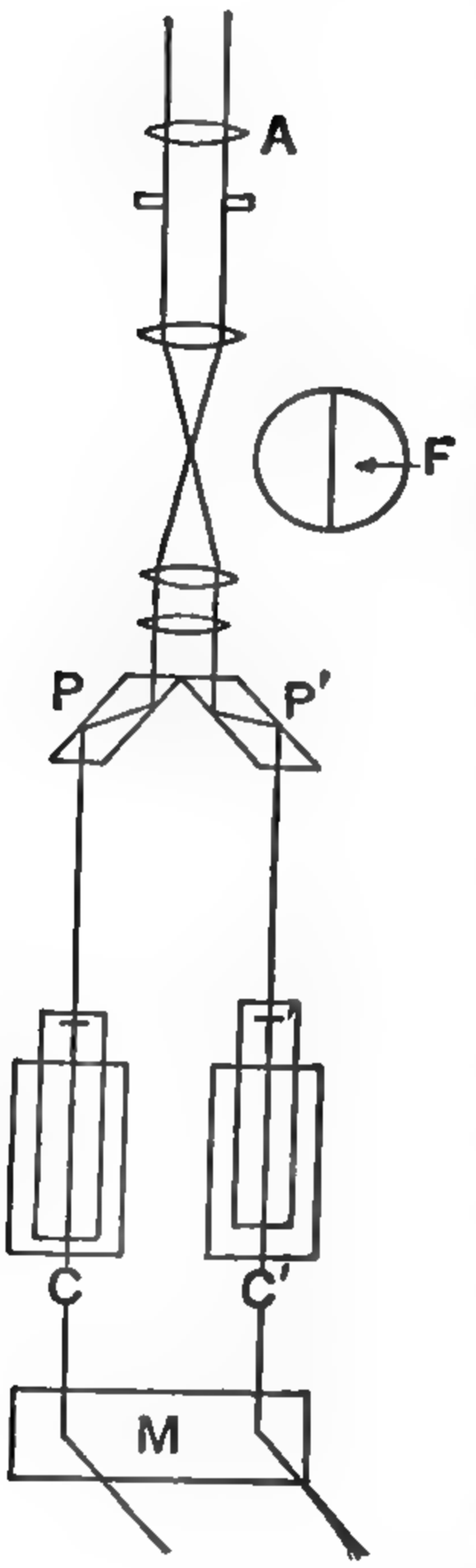


FIG. 240. Showing construction of Duboscq colorimeter.

Before the instrument is used the zero points of the scales should be checked by raising the cups until the lower surface of the plunger strikes the bottom of the cup. If necessary, the vernier is adjusted to zero. The cups are removed, the instrument is turned toward the light, and its position as well as that of the mirror is adjusted in such a way that the two halves of the field show equal brightness and color.

The cups are filled about half full, one with the sample, the other with the standard, replaced in the instrument, and raised until the plungers are well immersed in the liquid. If any air bubbles collect on the lower surface of the plunger the instrument is slightly tilted to remove them. The depth of the sample liquid is now fixed at a definite point on the scale, preferably an exact number of millimeters. The cup with the standard solution is raised or lowered until an exact match is obtained in the field, and the corresponding scale is read. The readings are repeated with different settings of the sample cup. Then the concentration of the sample liquid is calculated by

means of the formula given above, for each of the different experiments, and the results are averaged.

Example. A cane sirup has been treated with a chemical, such, for instance, as magnesium oxide, and it is desired to know how much coloring matter has been removed by the process. The original sirup, the color concentration of which will be called 100, is poured into one cup of the colorimeter, the treated sirup in the second, and upon matching the following readings are obtained:

Original Sirup	Treated Sirup	Computation
50 mm.	74.2 mm.	$50 \times 100/74.2 = 67.4$
40 mm.	59.2 mm.	$40 \times 100/59.2 = 67.6$
35 mm.	52.1 mm.	$35 \times 100/52.1 = 67.2$
		Average 67.4

The concentration of coloring matter in the treated sirup averages 67.4 per cent of that in the original, indicating a decolorization of 32.6 per cent.

Stammer's Colorimeter. Colorimeters are employed in technical sugar analysis for grading sirups, for estimating the decolorizing power of bone black or other clarifying agent, as shown in the above example, and for many other purposes. The absolute concentration of coloring matter in sugar products is unknown, and, in order to compare colorimetric measurements made at different times or in different laboratories, it is therefore necessary to employ a reproducible color standard. Stammer introduced disks of colored glass for this purpose, and designed a colorimeter which is extensively used in the sugar industry[92] (Fig. 241). The general principle of this apparatus is the same as that of Duboscq. The liquid to be tested is placed in the cylinder *a*, which is closed by a glass plate at the bottom. The measuring tube *c*, also closed at the bottom by a glass plate, fits loosely into *a* and can be raised or lowered to any desired level. The comparison tube *b*, which is open at the bottom, is joined to *c*, the two being moved in conjunction by a slide in the back of the instrument. The colorimeter is illuminated by a reflector at the bottom, the light passing upward through *b* and *c* into the prisms in *d* which produce the same double-field effect as in the Duboscq apparatus.

Daylight as a source of illumination produces discrepant results because of large variations in intensity. It is advisable to use artificial white diffuse light, such as that from a frosted bulb, placed in a fixed position relative to the colorimeter. If the current fluctuates considerably, as often happens in a factory, an adjuster to produce constant intensity should be used.[93] A movable diaphragm is inserted between

[92] Stammer's "Zuckerfabrikation," p. 747, 1887.

[93] Sázavský, *Z. Zuckerind. čechoslovak. Rep.*, 53, 147 (1928/29).

the light source and the instrument to produce exactly equal brightness in the two halves of the field.

In operating the colorimeter the standard plate of colored glass is placed upon tube *b*, which together with tube *c* is then raised or lowered until the intensity of shade for solution and color plate is the same in both halves of the field. A millimeter scale upon the back of the instrument marks the elevation of the measuring tube above the bottom of the cylinder, thus indicating the thickness of the column of liquid.

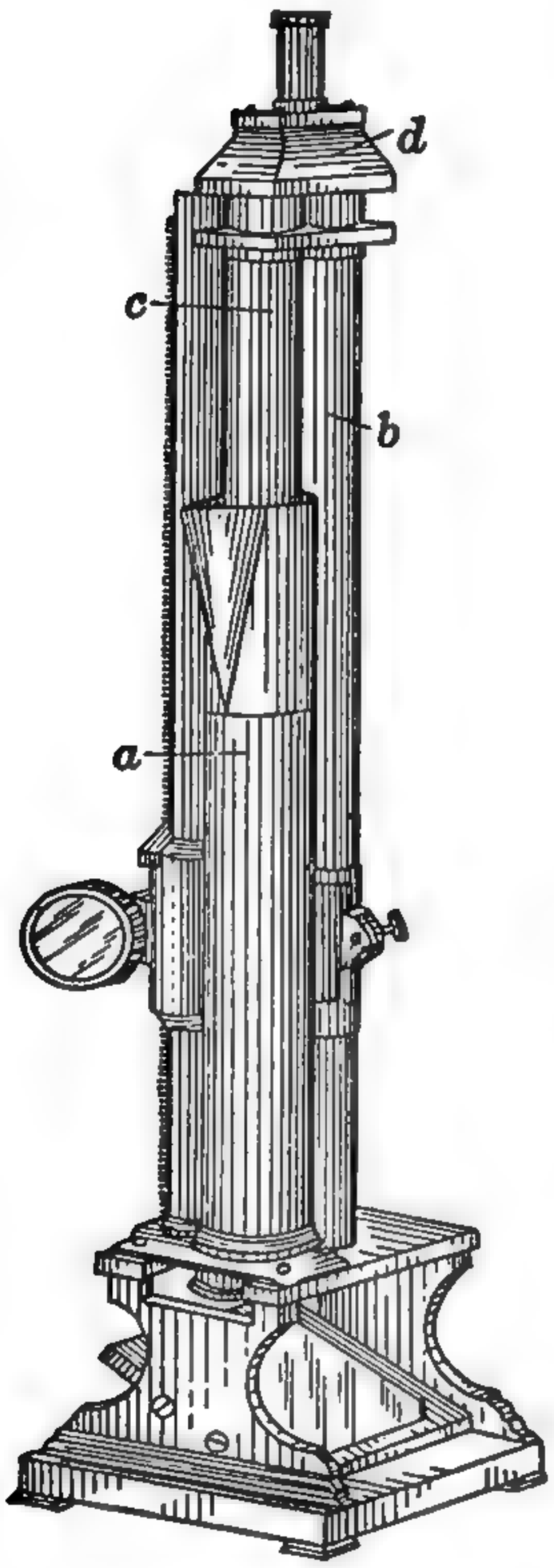


FIG. 241. Stammer's colorimeter.

If too much liquid is placed in the cylinder it will run over and soil the instrument. This may be obviated by attaching an overflow tube to the lip of the cylinder, as proposed by Kopperl.[94] Another side tube, with a petcock, is mounted directly above the bottom of the cylinder, to withdraw the liquid and wash water, without removing the cylinder. If another determination is to be made immediately, the cylinder and plunger are rinsed with the next solution, and the cylinder is then filled with it.

Stammer gives a solution which matches the standard plate for a scale reading of 1 mm., a color value of 100. The color value of any liquid in Stammer degrees is found by dividing 100 by the reading of the scale in millimeters.

In measuring the color of sugars, molasses, etc., a weighed amount of substance is dissolved in water, made up to a definite volume, and, if the solution is not clear, filtered. The color value of the solution is then calculated either to the original amount of substance, or to a polarization of 100, according to requirement.

Example. Twenty grams of a sugar, polarizing 92.4, was dissolved to 100 ml. and filtered. The solution gave a reading of 15 mm. upon Stammer's colorimeter. Then $100 \div 15 = 6.666$, the color value of the solution. The color value calculated to 100 parts sugar would be $20 : 6.666 :: 100 : x = 33.33$. The latter calculated to 100 polarization would give $92.4 : 33.33 :: 100 : x = 36.07$.

[94] *Deut. Zuckerind.*, **56**, 1037 (1931).

A table of reciprocals (Appendix, Table 11) will be found convenient for converting the scale measurements of Stammer's colorimeter into color units.

When the amounts of coloring matter in sugar solutions of different concentration are to be compared the results are expressed in Stammer degrees, on the basis of 100 g. dry substance in 100 ml. of solution. The calculation is made by the formula:

$$\text{Stammer degrees (° St.)} = \frac{100 \times 100}{h \times c}$$

where h is the reading, in millimeters, on the Stammer colorimeter, and c is the percentage concentration (Brix × density).

Measurements with the Stammer colorimeter are subject to two serious sources of error. Many of the normal and half-normal standard glasses furnished by the manufacturers differ widely from Stammer's original standard. It is therefore necessary first to test the glasses to be used, or to have them tested in an official bureau of standardization, by spectrophotometric analysis. The normal standard glass should show the characteristics given by Spengler and Landt,[95] and reproduced in Table LXXXII. The first column shows the wavelength in millimicrons, the second the transmission in fractions of unity, and the third the negative logarithms of the transmission, which are directly proportional to the color depth.

TABLE LXXXII

SPECTROPHOTOMETRIC ANALYSIS OF THE NORMAL STAMMER GLASS

Wavelength	Transmission	Negative Log of Transmission	Wavelength	Transmission	Negative Log of Transmission
mμ			mμ		
460	0.1493	0.826	540	0.5321	0.274
470	0.1879	0.726	560	0.6138	0.212
480	0.2344	0.630	580	0.6561	0.183
490	0.2844	0.546	600	0.6653	0.177
500	0.3467	0.460	620	0.6823	0.166
520	0.4436	0.353	640	0.6745	0.171

The negative logarithms of the transmission of the half-normal glass should be one-half of the figures given in the table.

Another method for checking the Stammer glasses, and for applying corrections in the event of deviations from the standard, has been

[95] *Z. Ver. deut. Zucker-Ind.*, **83**, 223 (1933).

introduced by Šandera.[96] It consists of a comparison with a standard solution which has practically the same absorption curve as the Stammer glass. This solution is prepared by dissolving 1.000 g. nickel ammonium sulfate [$NiSO_4$, $(NH_4)_2SO_4 \cdot 6H_2O$], 1.200 g. cobalt ammonium sulfate [$CoSO_4$, $(NH_4)_2SO_4 \cdot 6H_2O$], and 0.019 g. potassium dichromate in dust-free, carefully distilled water, and diluting to 100 ml. The nickel and cobalt salts are first recrystallized from water and dried in dust-free air on a glass plate. The recrystallized potassium dichromate is dried at 150° C. These chemicals are stored in glass-stoppered bottles, and the solutions are prepared fresh as needed. This standard solution has a color of exactly 5° Stammer, that is, 1° Stammer is defined by a thickness of 20 mm. of the solution. If a normal glass to be checked is found to be matched in the colorimeter by, e.g., 22.8 mm. of the standard solution, then all the results obtained with this glass must be multiplied by $22.8 \div 20, = 1.14$, to convert the degrees found into standard Stammer degrees.

The second of the sources of error mentioned above is the fact that the tint of the solutions to be measured often differs appreciably from that of the Stammer glass. This is particularly true of cane products. Such differences in tint make it very difficult or impossible to match the brightness of the two halves of the field. It then becomes necessary to resort to the use of monochromatic light, such as that supplied by a sodium or mercury vapor lamp, with appropriate filters.

Spectrocolorimeter of Spengler and Landt.[97] This is essentially a Duboscq colorimeter in which monochromatic light is furnished by two dispersion prisms and a collimator slit. This monochromator is constructed in such a way that only light of wavelengths 610, 560, or 480 mμ is allowed to pass. The instrument is used as a Stammer colorimeter by filling the solution to be measured into both cups, inserting a normal Stammer plate on one side of the field, and matching at one or more of the three wavelengths. If the height of the solution on the side without the Stammer glass is designated by a, and that of the solution on the side with the Stammer glass by b, then the difference between the two heights is equivalent to the Stammer glass, and the color value of the solution is calculated by the formula $100/(a - b)$. The color values found at the different wavelengths are usually not the same, and therefore the wavelength at which the measurements were made must always be specified. The ratios between the Stammer degrees found at the three wavelengths are indicative of the quality of the coloring matter.

[96] *Z. Zuckerind. čechoslovak. Rep.*, **57, 44 (1932/33)**.
[97] *Z. Ver. deut. Zucker-Ind.*, **81, 13 (1931)**.

The Spengler and Landt instrument may also be used as an ordinary colorimeter, for comparing solutions of unknown color concentration with a given standard solution.

The Lovibond tintometer,[98] used extensively in the malt industry, and the Pfundt colorimeter,[99] designed for grading honey according to color, are also employed to some extent for examining sugars and sirups.

Determination of the Decolorizing Power of Chars. This is accomplished by measuring the color of a sugar or molasses solution before and after treatment with bone black or activated carbon.

Bone Black. Meade[100] describes the following method for laboratory tests with bone black:

A cylindrical copper vessel, 4 inches in diameter by 15 inches high, is used to hold the char. Each vessel should be provided with a small cock at the bottom. The char should rest upon a perforated copper plate covered with cloth. The filters should be immersed in a water bath, provided with suitable openings for the outlet cocks, and should be filled to within a few inches from the top with the chars to be compared. The weight of the char should be the same in each of the filters. A suitable solution for comparisons is prepared by dissolving a molasses sugar to form a liquor of about 55° Brix, clarified by filtration with kieselguhr as in the refinery. This liquor should be heated to about 165° F., and equal portions of it should be added to each filter, little by little, or as under service conditions, to avoid forming air pockets. After covering the char, the remainder of the liquor may be poured into the filter. The temperature of the water bath is maintained at 160° to 170° F. for several hours, and then the filtered liquor is drawn from the outlet cocks. The color of the filtrate is then compared with that of the original liquor in a Stammer or other colorimeter, and the decolorization calculated. If the solutions are too dark for colorimeter readings, they are diluted with water to the same density.

Example. An unfiltered sirup diluted to 10° Brix gave a reading of 8 mm., or $\frac{100}{8} = 12.5$ color units, using a Stammer colorimeter. The liquid, after filtering through bone black and diluting to 10° Brix, gave a reading of 40 mm., or $\frac{100}{40} = 2.5$ color units. The amount of color removed by the bone black is then $\frac{12.5 - 2.5}{12.5} \times 100 = 80$ per cent.

If information on the relative removal of different coloring substances in sugar liquors is desired, a partial or complete spectropho-

[98] Bardorf and Ball, "The Elements of Sugar Refining," p. 220, 1925.

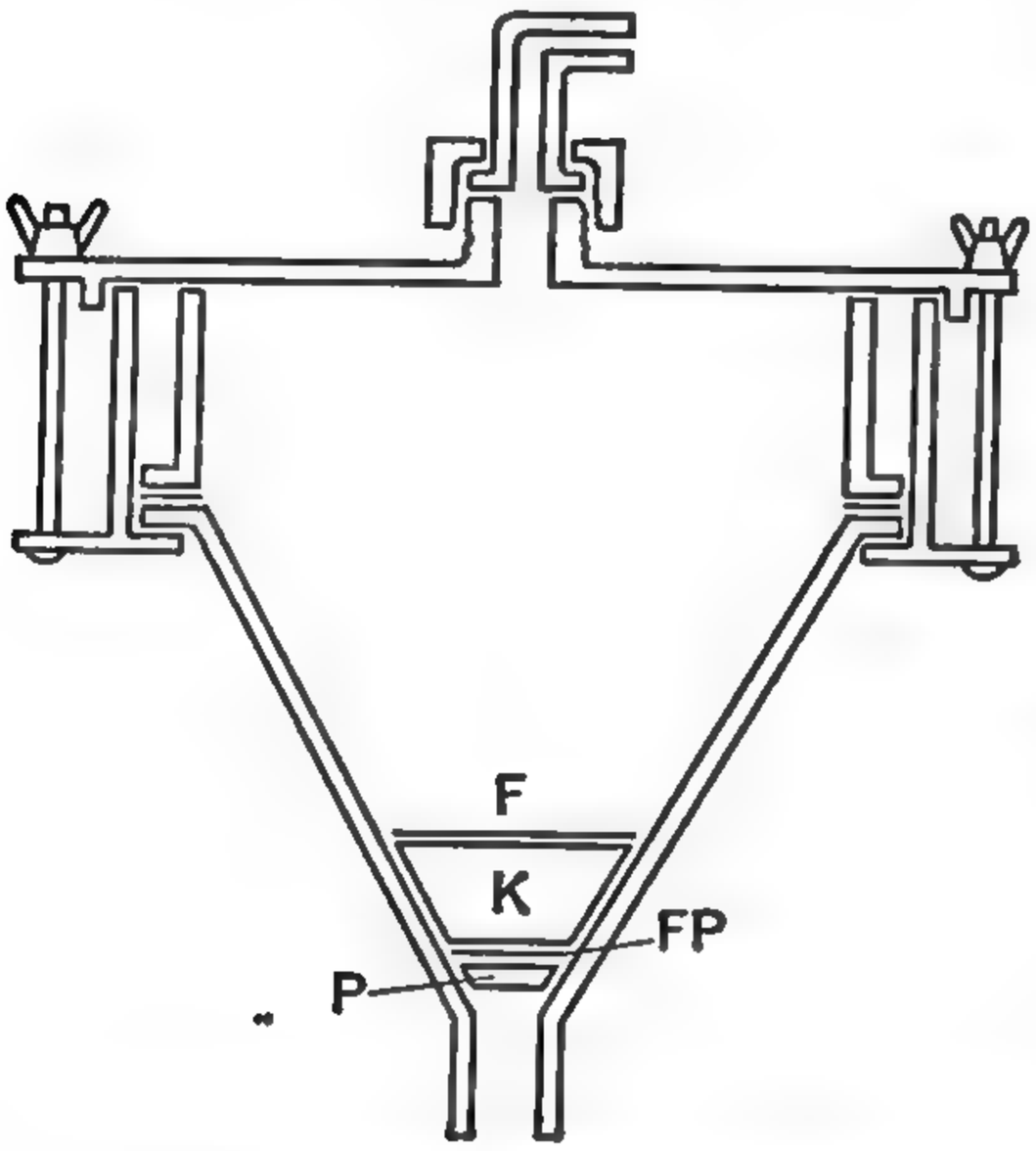
[99] Willaman, *Ind. Eng. Chem.*, 20, 701 (1928); Willaman and Easter, *Ind. Eng. Chem.*, 21, 1138 (1929); Balch, *Ind. Eng. Chem.*, 22, 255 (1930).

[100] "Spencer's Handbook for Cane-Sugar Manufacturers," 7th ed. by Meade, p. 187, 1929.

tometric analysis of both the original and the decolorized solutions must be made.

Another filtration method, based on the same principle as that of Meade, has been proposed for use in beet-sugar refineries by Šandera and Mirčev.[101]

Activated Carbons. Two different procedures are used in decolorizing sugar liquors on a factory scale with activated carbons. Some refineries filter a suspension of the carbon in water through presses, and then pass the liquor through the carbon bed (layer method); others mix the carbon directly with the liquor and filter off the carbon (mixing method). In either case the laboratory tests should duplicate these conditions as closely as possible.



(Reproduced from Z. Zuckerind. čechoslovak. Rep., 54, 260.)

FIG. 242. Edelstein's apparatus for testing the decolorizing power of activated carbon.

An apparatus for testing the decolorizing power of carbon used according to the layer method has been designed by Edelstein,[102] and is shown diagrammatically in Fig. 242. It is constructed of heavy metal to withstand high pressure (45 to 75 pounds). *P* is a Hirsch filter plate of 15-mm. diameter, cemented into the funnel. Above this is placed a circle of fine metal gauze which supports the filter paper *FP*. One gram of the carbon to be tested is shaken up with water, the suspension poured on the paper, the apparatus closed, and the water filtered off under pressure. The first cloudy portions of the filtrate are returned to put the carbon quantitatively on the filter. Then a piece of cloth *F* is laid over the carbon layer *K*, and fastened by means of a metal ring pressed down to prevent whirling of the carbon when the liquid to be decolorized is added. A solution containing 100 g. of molasses per liter is prepared at room temperature, and prefiltered cold with kieselguhr, to remove suspended matter. Two hundred milliliters of the solution is poured into the funnel and filtered under pressure. If any carbon should run through, the filtrate must not be poured back, because a second filtration increases the decolorization;

[101] *Z. Zuckerind. čechoslovak. Rep.*, **58**, 177, 185 (1933/34).
[102] *Z. Zuckerind. čechoslovak. Rep.*, **54**, 257 (1929/30); **56**, 49 (1931/32).

the carbon must be removed by filtration through paper. The clear filtrate is then compared colorimetrically with the original solution filtered through kieselguhr. The standard molasses solution must always be freshly prepared and must not be heated. All comparative tests must be made with an apparatus of the same dimensions, with the same molasses, filter paper, and cloth, at the same temperature, pressure, and hydrogen-ion concentration, because all these factors influence the results decidedly.

If the mixing method is practiced in the refinery, the laboratory test is carried out by adding a weighed quantity of carbon to a definite volume, say 200 ml., of a molasses solution prefiltered by means of kieselguhr. The mixture is heated with stirring at a definite temperature, say 80° C., in a water bath for a definite time, say 10 minutes. The solution is then quickly cooled to room temperature and filtered through filter paper. In comparative tests the chosen conditions must be strictly adhered to. The absolutely clear filtrates are compared with the original solution prefiltered through kieselguhr, and the decolorization calculated.

The relative decolorizing power of two carbons is not a fixed quantity. Even with the same sugar liquor the relative efficiency of two carbons cannot be determined by comparing the decolorizing effect of equal weights of the carbons, or by ascertaining how much of each is required to bring about a certain percentage decolorization. This is due to the fact that decolorization by carbons is an adsorption phenomenon and satisfies the Freundlich adsorption equation:

$$\frac{x}{m} = kc^{1/n}$$

where x is the amount of coloring matter adsorbed from a given volume of solution, m the number of grams of carbon used, c the concentration of the coloring matter in the decolorized filtrate, and k and $1/n$ are constants. The formula may also be written in the following logarithmic form:

$$\log\left(\frac{x}{m}\right) = \frac{1}{n}\log c + \log k$$

which is a straight-line equation. · To construct the complete decolorization curve of a carbon, tests as described above are made with increasing quantities of carbon, say 0.5, 1, 1.5, 2.0, etc., to 5 g. of carbon, added to 200 ml. of molasses solution. The concentration of coloring matter is expressed in any convenient unit, Stammer degrees, Meade-Harris units, or as —log t (see p. 593). The values of log (x/m) are

then plotted on cross-section paper against those of log c. From the straight line thus obtained the values of log k and of $1/n$ can be read off, log k being the value of log (x/m) for $c = 1$ ($\log c = 0$), while $1/n$ is the tangent of the angle of inclination of the adsorption curve. k and $1/n$ may also be computed mathematically from the values of x/m and c. They completely characterize the decolorizing effect of the carbon for the solution experimented with. Since the values of the constants vary not only with the carbon used but also with the product to be decolorized, it is best in practice to determine the decolorization curve for the particular liquor or other product which is to be decolorized.

Zerban[103] has found that sometimes the decolorization curve of one carbon will intersect that of another at a given point. This means that, when equal quantities of carbons are used, carbon A will do better than carbon B up to the point of intersection, while beyond that point carbon B will be better.

Spectrophotometry

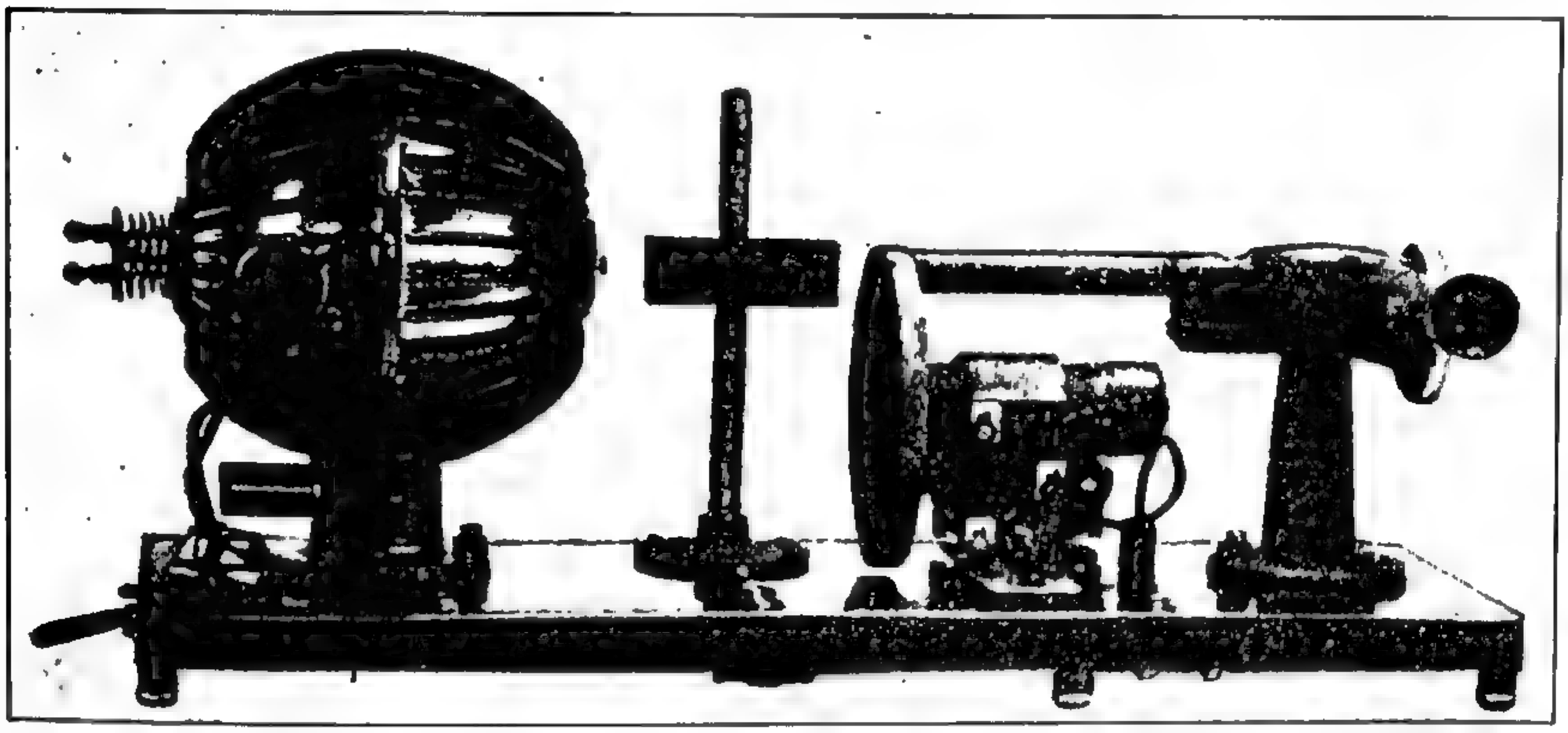
It has been pointed out that the use of Stammer glasses and similar arbitrary standards is unsatisfactory because, even if monochromatic light is used, it does not permit of absolute measurements. Since about 1920, investigators in various countries have been engaged in a fundamental study of color and coloring matter in sugar products, and it has been found necessary to resort to spectrophotometry for the solution of the problems of the sugar chemist. This has made it possible to express the concentration of coloring matter, as well as its quality, in physical units.

The Spectrophotometer. This instrument, as its name implies, is a combination of a spectrometer and a photometer. The purpose of the spectrometer is to furnish substantially monochromatic light by spectral dispersion of the white light used as a source, and at the same time to specify the wavelength. The photometer measures the fraction of the incident monochromatic light which is either transmitted by a colored medium or reflected by a colored surface.

The Keuffel and Esser Color Analyzer. This spectrophotometer, designed by Carl W. Keuffel, is widely used in the United States. Figure 243*a* shows, from left to right, the lamp house, the sample holder for measurements by transmitted light, the motor-driven photometer, and the spectrometer. The construction of the instrument may be seen in Fig. 243*b*. The spectrometer is of the constant-deviation type. The prism (19) is rotated by wavelength drum (4) to furnish monochromatic light of any desired wavelength between 430 and 700 mμ. The

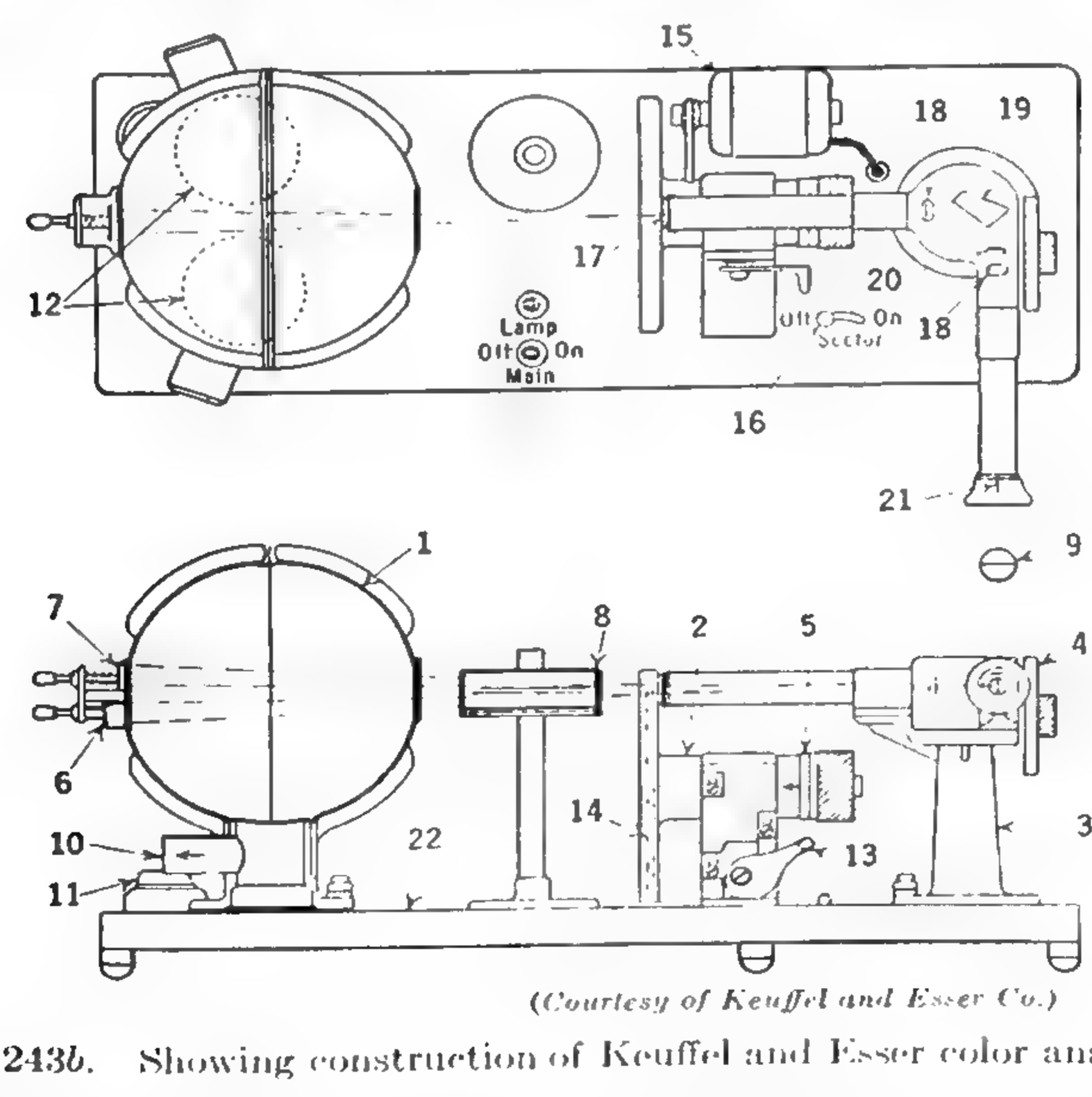
[103] *Facts About Sugar*, 13, 211 (1921).

biprism (20) divides the field into two equal parts. The width of the entrance slit (17) can be varied to provide for greater or less illumina-



(Courtesy of Keuffel and Esser Co.)

FIG. 243*a*. Keuffel and Esser color analyzer.

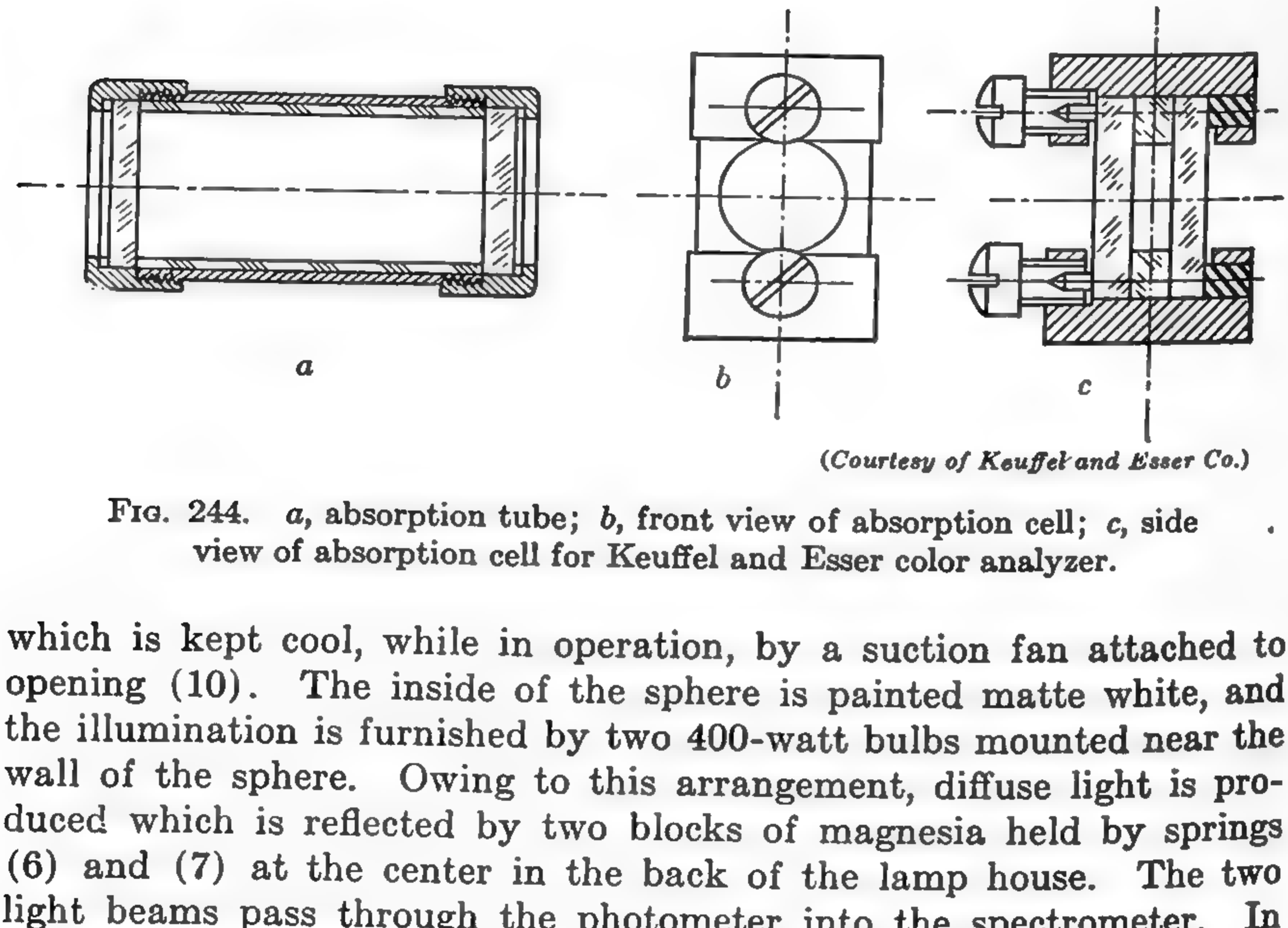


(Courtesy of Keuffel and Esser Co.)

FIG. 243*b*. Showing construction of Keuffel and Esser color analyzer.

tion; the narrower the slit, the greater is the spectral purity of the light, but at the expense of light intensity. The photometer consists of two sectored disks which are rapidly rotated by motor (15). They are

constructed in such a way that, while the motor is running, the intensity of the lower beam of light used as the standard of comparison can be varied from 0 per cent (complete darkness) to 100 per cent (standard maximum light intensity) and beyond to 110 per cent so as to be able to approach the 100 point from both sides for accurate settings. The photometer is also provided with a device which lowers the center line of the sectors; when this is used the readings must be divided by 4. This makes it possible to read dark-colored samples with greater accuracy. Sample holder (8) is used for measurements by transmitted light. Colored glasses and similar objects are held by means of a spring. Solutions are placed in tubes or cells of known thickness, shown in Fig. 244. The lamp house (1) is in the form of a sphere



(Courtesy of Keuffel and Esser Co.)

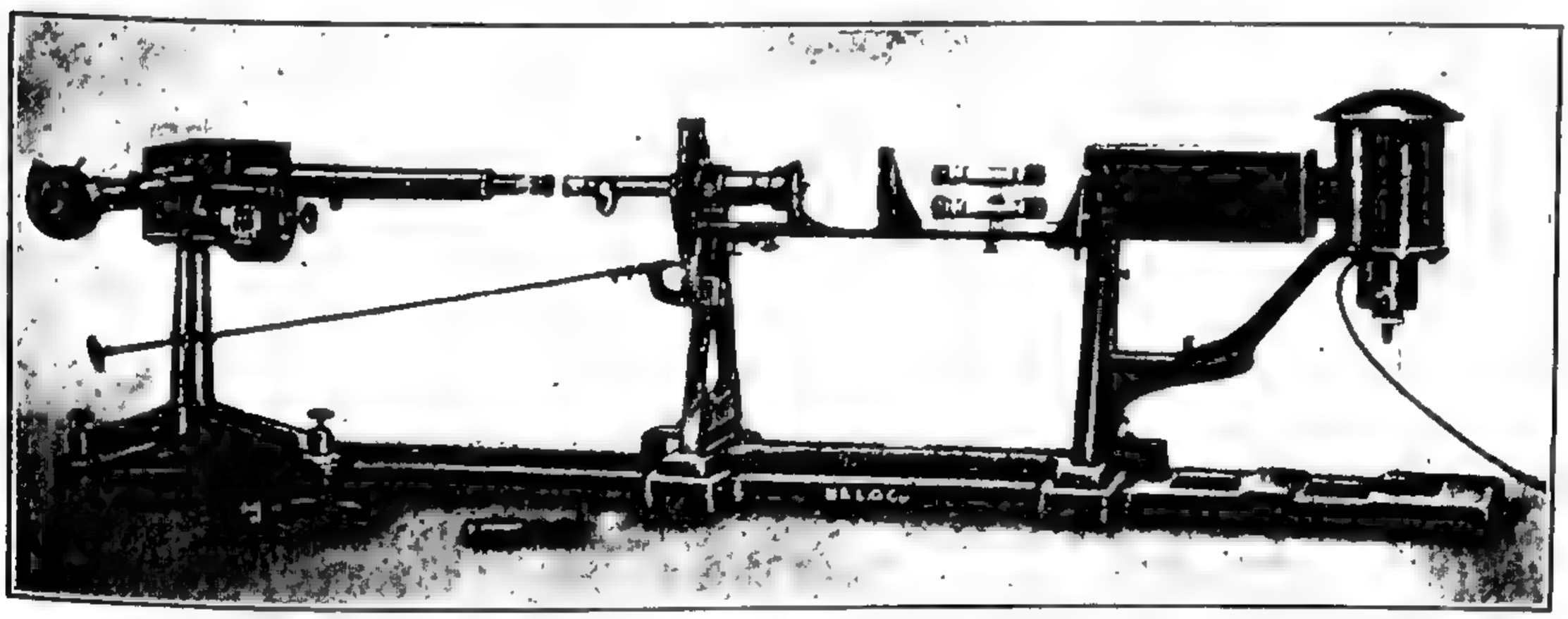
FIG. 244. *a*, absorption tube; *b*, front view of absorption cell; *c*, side view of absorption cell for Keuffel and Esser color analyzer.

which is kept cool, while in operation, by a suction fan attached to opening (10). The inside of the sphere is painted matte white, and the illumination is furnished by two 400-watt bulbs mounted near the wall of the sphere. Owing to this arrangement, diffuse light is produced which is reflected by two blocks of magnesia held by springs (6) and (7) at the center in the back of the lamp house. The two light beams pass through the photometer into the spectrometer. In the case of reflectance measurements the sample is substituted for the magnesia block in holder (7). Before the instrument is used, the settings of both the spectrometer and photometer must be carefully tested. To check the wavelength drum, a monochromatic light source, such as a sodium flame, helium tube, etc., is used, and if necessary the drum or the prism is adjusted so that the particular spectral line used appears in the center of the field of vision. On the photometer drum the 0 and 100 points must be verified. At the 0 point the upper half of

the field which, owing to inversion of the image, corresponds to the lower beam of light entering the photometer, must show maximum darkness; the adjustment is made on the photometer drum. At the 100 point the two halves of the field must appear equally bright; if they do not, adjustment is made by varying the relative slit width of the two halves of the entrance slit (17). Another good way to ascertain whether the instrument is in perfect adjustment is to insert a colored-glass plate of known transmission values in the upper beam; the readings must check those certified by some official testing laboratory as the Bureau of Standards.

Other spectrophotometers differ from the one described mostly in the design of the photometer and in constructional details of the spectrometer and light source. In some of them the photometric device is based on Vierordt's principle of varying the light intensity by changing the width of the entrance slits through a micrometer arrangement. The slits may be placed either directly above each other, as in the Keuffel and Esser instrument, or else they may be placed in two different collimator tubes. In other types the intensity of the comparison beam is varied by means of a polarizing system and an analyzing Nicol prism.

Spectrophotometer with Polarization Photometer. Of the various instruments of this type only one will be described as an example.[104]



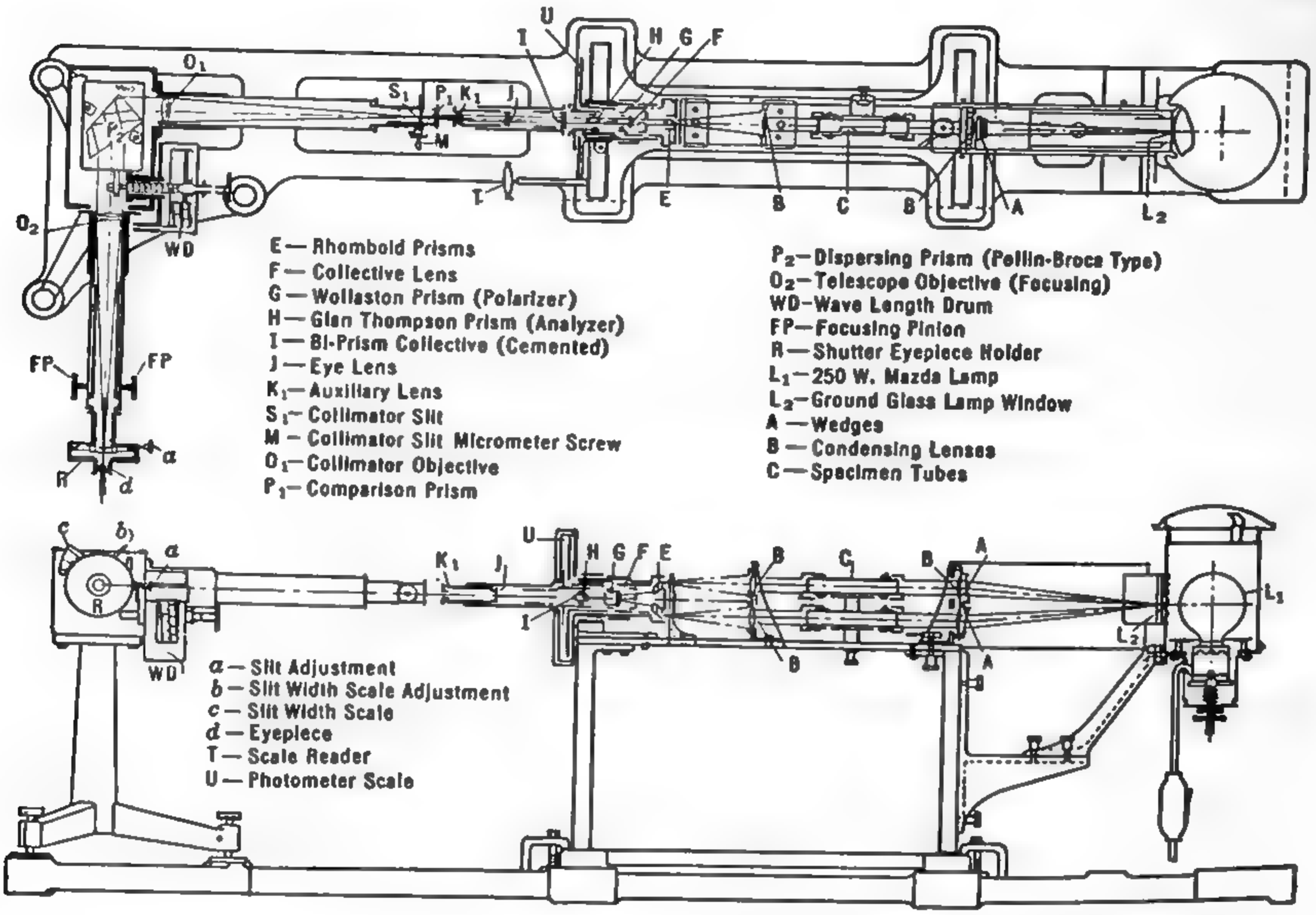
(Courtesy of Bausch and Lomb Optical Co.)

FIG. 245*a*. Bausch and Lomb spectrophotometer.

A general view of it is given in Fig. 245*a*, and the optical system is shown diagrammatically in Fig. 245*b*. White light is furnished by a

[104] Manufactured by Bausch and Lomb Optical Co.; similar instruments are made by Gaertner Scientific Corp.; Adam Hilger, Ltd.; Schmidt and Haensch, and others.

250-watt Mazda lamp L_1, mounted in a cylindrical housing, and is diffused by a double ground-glass window L_2. If reflectance measurements are to be made this light source is replaced by a spherical lamp house similar to that in the Keuffel and Esser Color Analyzer. The two absorption tubes, one for the sample and the other for the solvent, are placed in holders between the light source and a modified Martens photometer. The two incident beams of light are reflected by rhomboid prisms E, pass through collective lens F, and are polarized by the Wol-



(Courtesy of Bausch and Lomb Optical Co.)

FIG. 245*b*. Showing construction of Bausch and Lomb spectrophotometer.

laston prism G. The analyzer is a Glan-Thompson prism firmly connected with a disk which is rotated by means of a long rod operated from the front of the instrument. The scale U, at the periphery of the disk, is read by magnifying glass T. From the analyzer the beams pass through a biprism I and lenses J and K_1 into the collimator slit S_1, the width of which may be varied by means of micrometer screw M. The spectrometer is similar to that in the Keuffel and Esser Color Analyzer. The field forms a rectangular section, with a horizontal dividing line; the length and width of the field can be regulated by special devices. If desired, the instrument is furnished with the usual circular field, divided horizontally.

The spectrophotometric readings are made, as with the Keuffel and Esser instrument, by setting the wavelength drum to the desired wavelength, and turning the photometer circle until the two halves of the field match in brightness. In the polarization photometer there are two points of complete extinction of each field, and equal brightness occurs at four points 90° apart. Two quadrants of the circle carry a circular scale, the third reads directly in percentage transmission, and the fourth gives the density, i.e., the negative logarithm of the transmission. With no samples interposed in the beams, the circular degree scale gives equal brightness at 45° and 135°, the transmission scale at the 100 point, and the logarithmic scale at 0.

If the circular degree scale is used, the transmission T_1 is calculated, according to the law of Malus, as follows:

$$T_1 = \tan^2 \phi \cot^2 \theta_1$$

where ϕ is the angle at 100 per cent transmission (45°), and θ_1 is the angle, which must be larger than ϕ, at which a match is obtained with the sample in place.

If the absorption cells are reversed in the sample holder, another match is obtained at an angle θ_2, smaller than ϕ; in this case

$$T_2 = \cot^2 \phi \tan^2 \theta_2$$

By reading the sample in both positions, any effects due to inequality of illumination, or to polarization in the sample itself, are eliminated, and it is thus possible to calculate the transmission T by combining the the equations for T_1 and T_2:

$$T = \cot \theta_1 \tan \theta_2$$

When the transmission scale or the logarithmic scale is used, it is best also to reverse the absorption cells and read in both positions. The two readings are then averaged.

If it is desired to make transmittancy readings at varying thickness, the sample holder is removed and replaced by a special holder with two vertical cups and plungers, similar to those in the Duboscq colorimeter. The lamp house is elevated, the horizontal beam issuing from it is directed downward by a system of prisms and lenses, and the beams emerging from the cups are again made horizontal by another system of prisms and lenses and directed into the photometer.

Since monochromatic light is used in spectrophotometry, there is no difference in hue between the two halves of the field, and for this reason even color-blind persons can obtain correct results. But the

determination of complete transmission or reflection curves by visual spectrophotometry requires much time and is very fatiguing for the observer. To obviate this difficulty Hardy[105] has designed a photoelectric spectrophotometer, manufactured by the General Electric Co.,[106] which automatically records the complete transmission or reflection curve on a sheet of paper, wavelength for wavelength. Another photoelectric instrument, which has no recording mechanism, however, is made by the Coleman Electric Co.; spectrally dispersed light is obtained by means of two diffraction gratings instead of a prism. The light intensities are measured with a potentiometer.

Measurements upon Solutions by Transmitted Light. Two absorption cells or tubes, which must be exactly alike, are generally used for such measurements. One of these is filled with the colored solution, e.g., of a sugar product, and the other with the solvent, usually water. In the case of sugar products it is better to use a colorless sucrose solution of the same density as the sample. If no comparison cell were employed the readings obtained would represent merely transmission values, and the results would have to be corrected for the light absorbed by the cover glasses and the solvent, and for that reflected at the various intervening surfaces. With the use of the comparison cell the readings give *transmittancy* values for the colored solution directly.

For purposes of color specification in the physical sense, transmittancy readings are taken at a number of points throughout the visible spectrum, generally 10 to 20 mμ apart. The transmittancies observed are plotted on graph paper as ordinates and the wavelengths as abscissas. The resulting curve presents a color analysis of the sample, and this can be readily translated into any of the various systems of color specification. A similar curve is obtained when reflectance measurements of a colored surface are made.

The Law of Lambert-Beer. In order to convert the results of transmittancy measurements upon a solution into *concentration* of *coloring matter*, the law of Lambert-Beer is made use of. In simple language, this law states that, as the number of particles of coloring matter in a solution increases, each additional particle reduces the remaining intensity of the incident monochromatic light to the same fraction of the intensity. To give a concrete example, if 1 part of coloring matter in solution reduces the original intensity 1 at a certain wavelength to 0.8, then 2 parts of the same coloring matter reduce the original intensity to 0.8 of 0.8, or $0.8^2 = 0.64$; 3 parts reduce it to 0.8^3, or 0.512, and so on. It is readily seen that the proportion between the

[105] *J. Optical Soc. Am.*, **18**, 96 (1929).

[106] *Instruments*, **9**, 68 (1936).

number of parts (1, 2, and 3) is the same as that between the negative logarithms of 0.8, 0.64, and 0.512, which are respectively 0.09691, 0.19382, and 0.29073.

The number of "parts" of coloring matter may be varied in two ways, either by changing the thickness of a solution at constant concentration (Lambert's law) or by changing the concentration at constant thickness of solution (Beer's law).

Designating the transmittancy of a solution (transmission after correcting for reflection at the cell surfaces and absorption by the solvent) by T, and the specific transmissivity (transmittancy reduced to unit conditions of thickness and concentration) by t, the law may be expressed by the following formula:

$$T = t^{bc}$$

where b is the thickness (expressed in centimeters) and c is the concentration (in grams per milliliter). Since both T and t are expressed as fractions, the logarithmic form of the formula may be written as

$$-\log t = \frac{-\log T}{cb}$$

$-\log t$ is called the "specific absorptive index."

Example. The concentration of a solution of potassium dichromate is to be determined by spectrophotometry. It is necessary first to know T, t, and $-\log t$ for a solution of known concentration. A solution containing 0.01 g. per ml. of pure potassium dichromate was prepared. A reading of this solution in a 2-cm. cell at wavelength 529 mμ gave a T of 20.3 per cent, or 0.203. The specific absorptive index, $-\log t$, is therefcre $-\log$ of 0.203, divided by 2×0.01, or 0.69251/0.02, or 34.625. The solution of unknown strength, in a 1-cm. cell, at the same wavelength 529 mμ, gave a transmittancy of 81.9 per cent, or 0.819. The concentration of the solution is therefore found to be $-\log$ of 0.819, divided by 1×34.63, or 0.08672/34.625, or 0.0025 g. per ml. Repeating the measurements on both solutions at wavelength 541, the first solution gave $T = 0.336$, $-\log T = 0.47366$, $-\log t = 23.683$. For the second solution there was found $T = 0.8725$, $-\log T = 0.05923$, and c, as before, $= 0.0025$.

For any individual coloring matter the $-\log t$ at any wavelength is a constant, within the limits for which Beer's law holds. Consequently, there is a constant ratio between the $-\log t$ at one wavelength and that at any other specified wavelength, irrespective of the concentration or thickness of the solution examined.

The Extinction Coefficient. This term, commonly employed in the German literature, is the negative logarithm of the transmittancy

(—log T), reduced to 1-cm. thickness according to Lambert's law, but not to unit concentration.

Determination of the Quantity of Coloring Matter in Sugar Products. In order to measure the concentration of the coloring matter occurring in sugar products by the spectrophotometric method, two conditions must be fulfilled. First, the actual concentration of coloring matter in a chosen standard sugar product must be known, by determination through another method. Second, the ratio between —log t at one wavelength and that at another wavelength must be constant; otherwise the result of a determination of the concentration *c* obtained by measurements at one wavelength would be different from that determined at another wavelength. Neither of these conditions holds for sugar products, and thus far the only advantage of the spectrophotometric method over the ordinary colorimetric method would be the possibility of obtaining an exact match between the two halves of the field.

But Peters and Phelps,[107] through a spectrophotometric study of several hundred sugar products, have been able to devise a system of expressing the concentration of coloring matter in sugar products in terms of the specific absorptive index. Since the absolute concentration of coloring matter in any sugar product is unknown, Peters and Phelps decided to use for *c* in Lambert and Beer's law the concentration of *dry substance* in the product, expressed in grams per milliliter. As a consequence, —log t at each wavelength is constant only for one and the same sugar product, but usually different for other sugar products.

Peters and Phelps made transmittancy determinations throughout the visible spectrum, and then drew complete luminosity curves for each product, on the basis of the visibility and energy values at each wavelength at which determinations had been made. Thus the optical center of gravity of the luminosity curve of each product could be calculated by integration; the transmittancy at the optical center could also be calculated or read from a graph. The color, in the physical sense, could thus be specified. A similar study was made with a pure white sucrose chosen as a standard, and its optical center of gravity was found to be at wavelength 560 mμ. In this way it became possible to postulate that the optical center of gravity of the luminosity curve for unit concentration of the coloring matter present in any sugar product be at the same wavelength which is the optical center of gravity for the luminosity curve of the standard. Under these conditions Lambert-Beer's law can be applied, on the basis of the —log t of the standard sucrose at wavelength 560 mμ. This standard sucrose shows at each

[107] *Bur. Standards Tech. Paper* 338, 1927.

wavelength a specific absorptive index, —log t, which is termed an "absorption unit," —log t_1, for that wavelength. The numerical value of —log t_1 naturally varies from wavelength to wavelength, and each absorption unit applies only to a designated wavelength.

It must be clearly understood that all the —log t_1 values of the standard and all the —log t values of any sugar product are merely measures of absorption, and not of concentration of coloring matter, except at wavelength 560 mμ.

Unit of Coloring Matter. *The unit of coloring matter* is defined by Peters and Phelps as that quantity which evokes the sensation of *one color degree*. The latter represents the sum total absorption over the entire visible spectrum of the standard sucrose, that is, the sum total of its individual absorption units, and its stimulus is measured by the luminosity integral, over the visible spectrum, of the standard sucrose. The —log t_1 at wavelength 560 mμ of the standard sucrose is numerically equal to its absorption integral.

Therefore, if for any sugar product the —log t is determined at wavelength 560 mμ, the number of units of coloring matter contained in it is simply found by dividing this —log t by the —log t_1 of the standard sucrose at wavelength 560 mμ. The numerical value of this —log t_1 has been found by Peters and Phelps to be 0.00485. This unit of coloring matter has not come into general use, however, and there is really no need for it because the —log t at wavelength 560 mμ completely specifies the concentration of coloring matter, being directly proportional to the number of units of coloring matter.

If information is desired not only about the quantity of coloring matter present, but also on its nature, readings are made at one or more other appropriate points in the spectrum. Then the —log t values at these wavelengths are divided by the —log t at 560 mμ, and the resulting Q ratios are an indication of the *quality* of absorption. In cane products the blue end of the spectrum is usually the most important, as it gives an idea of the "yellowness" of the coloring matter. In some cases readings throughout the visible spectrum may be advisable to discover the possible presence of unusual coloring matters.

Other Sugar Color Units. Besides the specific absorptive index and the Stammer degree discussed on pp. 579 and 591, other color units have been proposed from time to time. The Meade-Harris unit[108] is the amount of coloring matter which causes an absorbency of 1 per cent (transmittancy 99 per cent), the depth of solution and the concentration of total solids not being specified. The negative logarithm of absorbency 1 is 0.00436. The Meade-Harris units can therefore be converted

[108] *Ind. Eng. Chem.*, 12, 686 (1920).

into —log t by multiplying by 0.00436, and reducing to unit thickness and concentration.

Meade and Harris used the same color units in all the different regions of the spectrum, and this is tantamount to a substantially gray scale for the standard. Peters and Phelps have shown that such a standard is not suitable for sugar products. Meade and Harris also proposed to average algebraically the color units found for a red, a green, and a blue screen, and to divide by 3, to express the "color" of the sample. A figure obtained in this way is meaningless, however, because only an integration of the luminosity curve based on transmittancies would serve this purpose, and even then the result would bear no direct relation to the quantity of coloring matter present.

In the cane-sugar industry many other empirical color units, based on arbitrary standards, are used, sometimes varying from one factory to another. This practice should be discouraged and the ensuing confusion avoided by expressing the concentration of coloring matter always in terms of the specific absorptive index (—log t) at wavelength 560 mμ.

Conversion of Absolute Color Measurements into Stammer Degrees. The Stammer degree has been used for such a long time in the beet-sugar industry, particularly in Europe, that it conveys a definite mental picture to the chemist. Those who are unfamiliar with modern methods of color measurement frequently desire, therefore, to have the results of such measurements translated into Stammer degrees. Landt and Hirschmüller[109] have shown that this conversion is not a simple matter, even if the comparison is based on an accepted standard Stammer glass, because of the asymmetric construction of the Stammer colorimeter. In order to facilitate the calculation, Landt and Hirschmüller postulate the following conditions: A colorimeter with two cups is used; one of these is filled with the colored solution, the other with the same quantity of a colorless sucrose solution of the same density as the colored solution, and a normal Stammer glass is interposed between the colorless solution and the ocular; monochromatic light is used for the illumination. Then the Stammer degrees *St*, on the basis of the concentration expressed in grams per milliliter, are found by the equation

$$St = \frac{1}{c} \times \frac{100}{h} \quad \text{or} \quad \frac{1}{c} \times \frac{10}{d}$$

where c is the concentration of dry substance in grams per milliliter, h

[109] *Deut. Zuckerind.*, **62**, 531 (1937).

the height of the solution in millimeters, and d the same in centimeters.

When the two halves of the field show equal brightness,

$$-\log T_g = kd$$

where T_g is the transmission of the standard normal Stammer glass (see p. 579) and k is the extinction coefficient (—log T for 1-cm. thickness) of the solution. Substituting the value of d from this equation in the first equation, we obtain

$$St = \frac{1}{c} \times \frac{10\,k}{-\log T_g} = \frac{10\,k}{c(-\log T_g)}$$

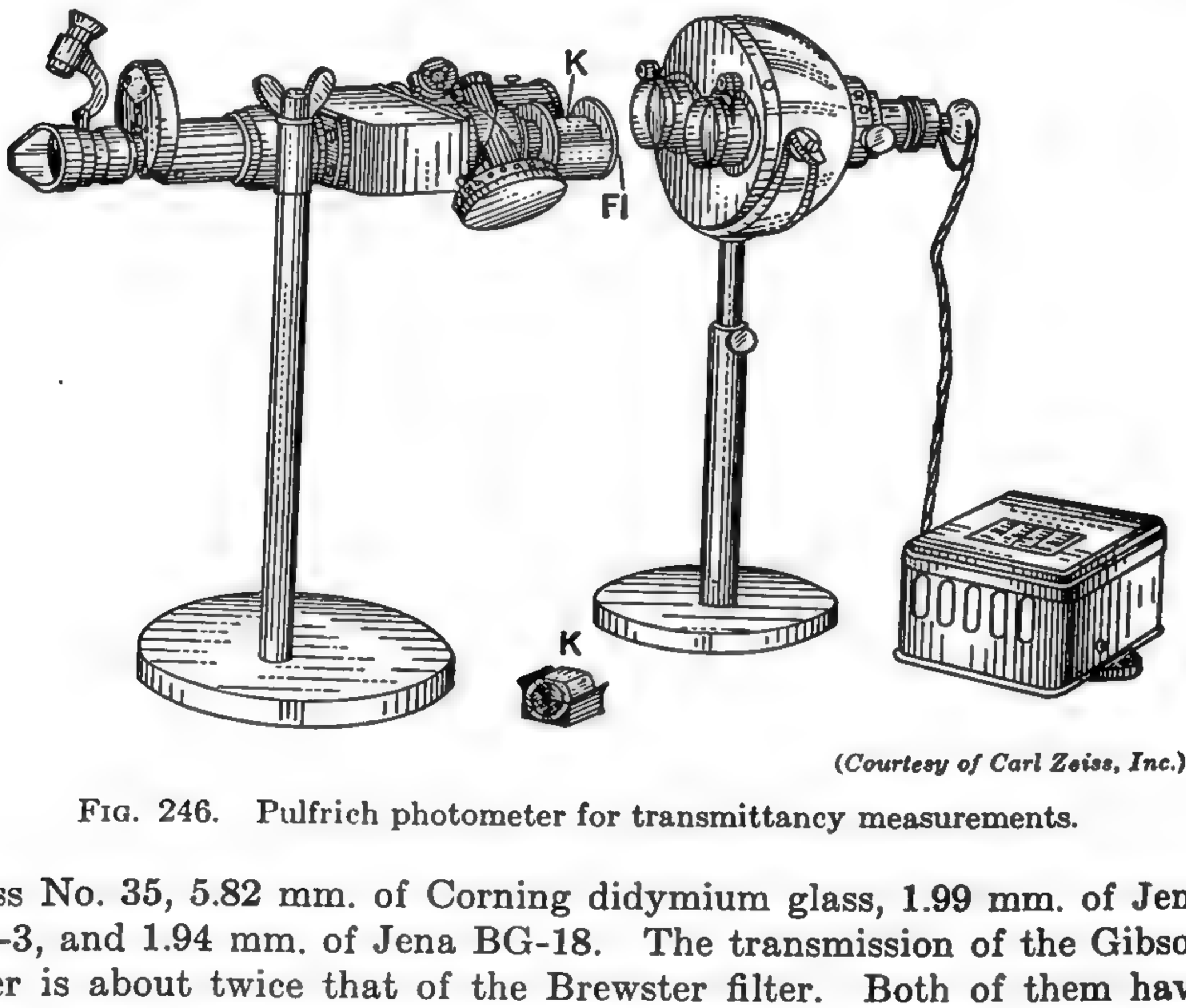
Since k/c equals the specific absorptive index —log t, the latter can be converted into Stammer degrees for the same wavelength by the simple equation

$$St = \frac{10(-\log t)}{-\log T_g}$$

If the calculation were to be made for the usual Stammer apparatus, corrections would have to be applied for losses due to reflection and to absorption by the solvent. But if this is done, the Stammer degrees found at different wavelengths for a dilute solution are not in the same proportion as those found for a more concentrated solution of the same colored product. The simple formula gives proportional Stammer degrees with varying concentration, and Landt and Hirschmüller conclude therefore that it is best to use it for the conversion. Actual measurements with the usual Stammer apparatus, under monochromatic light, would give values slightly different from those calculated, but the average of the Stammer degrees found at several wavelengths throughout the spectrum checks closely with those calculated, within the limit of error of the Stammer colorimeter. It is quite evident that this instrument is unsatisfactory for precise color measurements and should be abandoned in favor of the more modern methods.

Simplified Spectral Photometers. For many purposes it is not necessary to employ an expensive spectrophotometer, and a combination of any kind of photometer with a mercury-vapor lamp may suffice. The mercury lines at wavelengths 436, 546, and 578 mμ are screened out by appropriate filters, and readings taken either at the last two or all three wavelengths. The —log t at wavelength 560 may then be calculated, according to Peters and Phelps, by deducting, from —log t at 546, 48 per cent of the difference between —log t at 546 and 578 mμ. The reading at 436 mμ gives an idea of the quality of absorption. Additional monochromatic light sources, such as a sodium-vapor lamp, may also be used if desired.

Even the white light of a tungsten lamp may be employed, definite spectral bands being separated out by means of color screens. A filter with a narrow band and an effective wavelength of 560 mμ has been developed by Brewster.[110] It consists of one layer each of Wratten gelatin filters No. 21 and No. 61, cemented with Canada balsam between a 6.5-mm. layer of Corning didymium glass and 1 to 2 mm. of colorless glass. This cover plate serves to protect the gelatin films. The edges of the composite filter may be bound with black tape. Another filter for wavelength 560 mμ, devised by Gibson,[111] is made of 4.55 mm. of Corning



(Courtesy of Carl Zeiss, Inc.)

FIG. 246. Pulfrich photometer for transmittancy measurements.

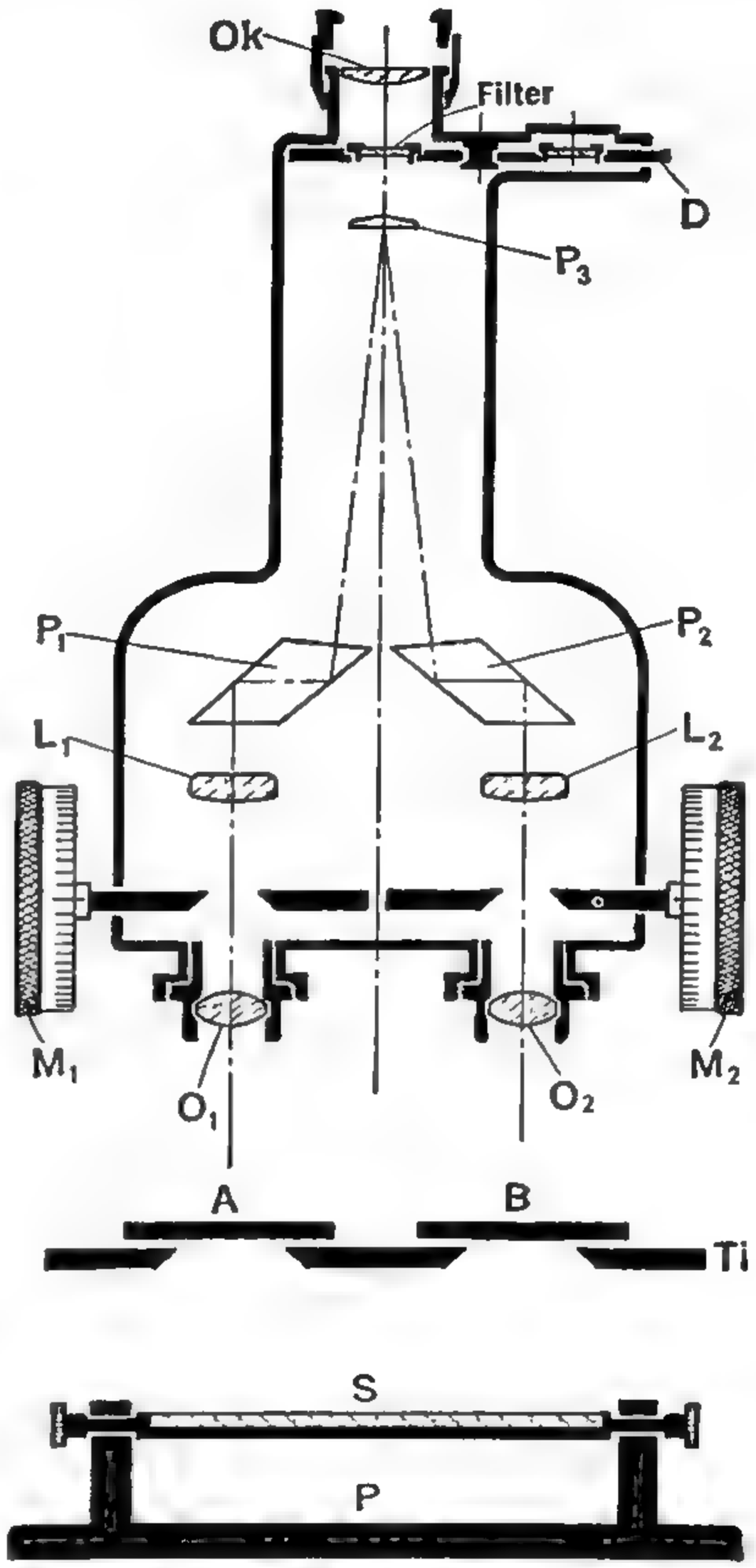
glass No. 35, 5.82 mm. of Corning didymium glass, 1.99 mm. of Jena VG-3, and 1.94 mm. of Jena BG-18. The transmission of the Gibson filter is about twice that of the Brewster filter. Both of them have been found to give satisfactory results in sugar colorimetry. They may be cut to such a size that they fit in the eyepiece of the instrument with which they are to be used. For color measurements on white sugars Brewster[112] recommends a filter with an effective wavelength of 460 mμ, consisting of Jena BG-12. Although wavelength 560 mμ should always be used when the concentration of coloring matter is to be determined, the practical difficulty arises with white sugars that very long absorp-

[110] *Facts About Sugar*, **28**, 228 (1933).
[111] *Bur. Standards J. Research*, **14**, 545 (1935).
[112] *Bur. Standards J. Research*, **16**, 349 (1936).

tion cells must be used in order to obtain reliable transmittancy readings. But at 460 mμ the transmittancy is low enough to be measured accurately with cells of moderate thickness.

Of the various photometers designed for a white light source, the instruments of Pulfrich and of Ives, which are used to some extent in sugar laboratories, will be briefly described.

The Pulfrich Photometer. This instrument, with its light source, is shown in Fig. 246 and the construction of the photometer diagrammatically in Fig. 247. At the objective end it has two openings with centers 7 cm. apart. Each of the two apertures is formed by two V-shaped shutters moving symmetrically in opposite directions, with their planes in direct contact (Fig. 248). The area of the aperture is varied and at the same time measured by means of drums mounted on the sides. The peripheries of the drums are calibrated in percentage transmission. The peculiar V-shaped construction of the shutters causes the percentage divisions to be much farther apart near the 0 point than near the 100 point. This makes it possible to read small transmissions with greater precision than when the percentage units are equidistant.



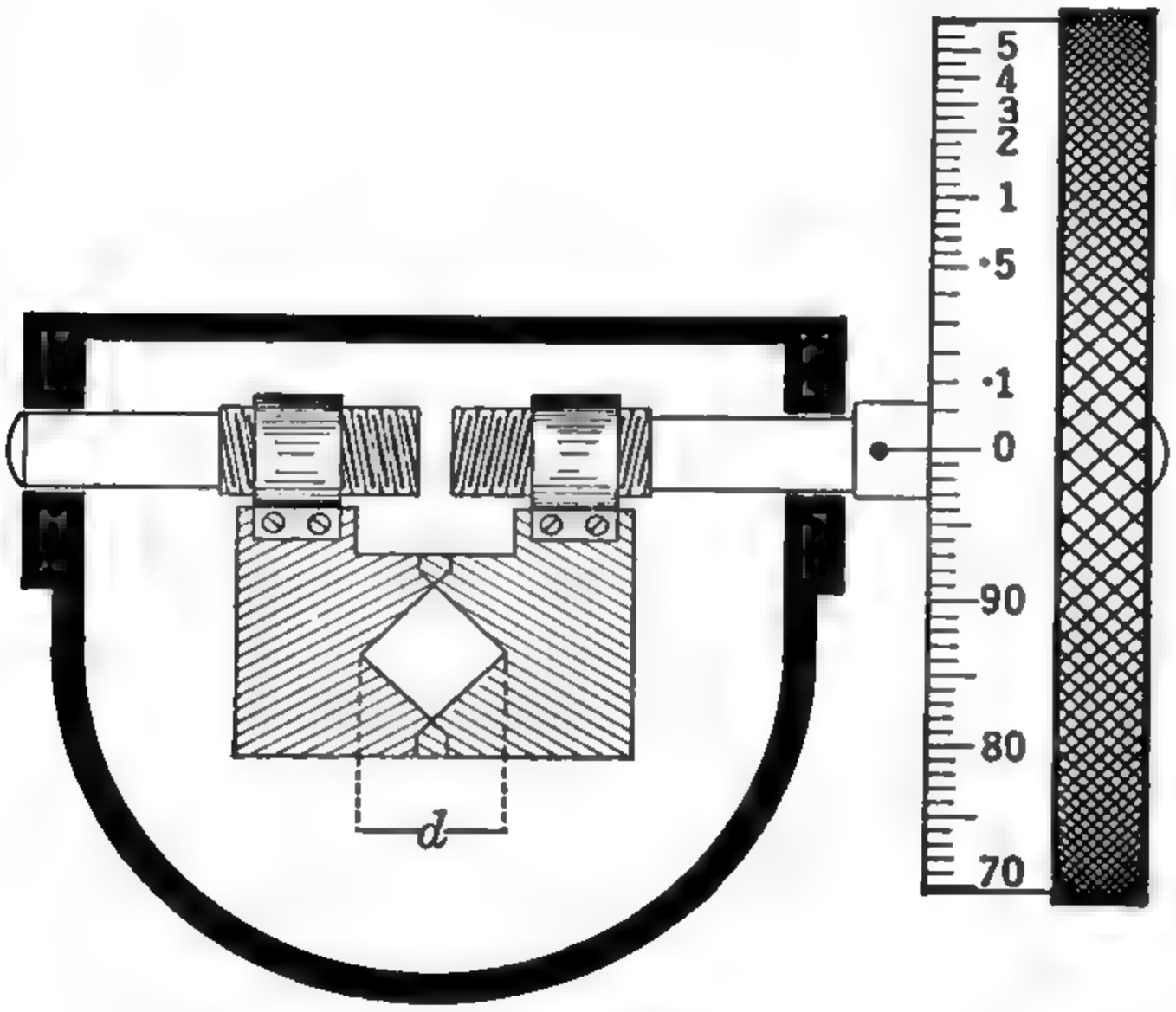
(*Courtesy of Carl Zeiss, Inc.*)

FIG. 247. Showing construction of Pulfrich photometer.

Since both apertures may be varied independently, either one may be used for measuring while the other is set at 100, and the two cells, one with the sample and the other with water, may be reversed. This makes it possible to detect any differences in the intensity of the two primary light beams from the lamp. The beams passing through the two apertures are brought into juxtaposition in the field of vision by a system of prisms and lenses. The color screens are

placed in a revolving drum mounted in the ocular, and they may be turned into the field in regular order. Eight color filters, covering the visible spectrum from the extreme blue to the extreme red end, are furnished with the instrument. Their effective wavelengths are 750, 720, 610, 570, 530, 500, 470, and 430 mμ.

Absorption cells up to 3-cm. thickness are placed in holders fastened to the rear of the photometer apertures. If longer absorption tubes are required, for readings of nearly colorless solutions, they are placed on a special stand between the photometer and the light source. The lamp house is hemispherical. It has two circular openings in front, with centers 7 cm. apart to correspond with the photometer apertures. It furnishes two parallel beams of equal intensity when properly adjusted. The light may be diffused by insertion of frosted-glass disks.



(*Courtesy of Carl Zeiss, Inc.*)

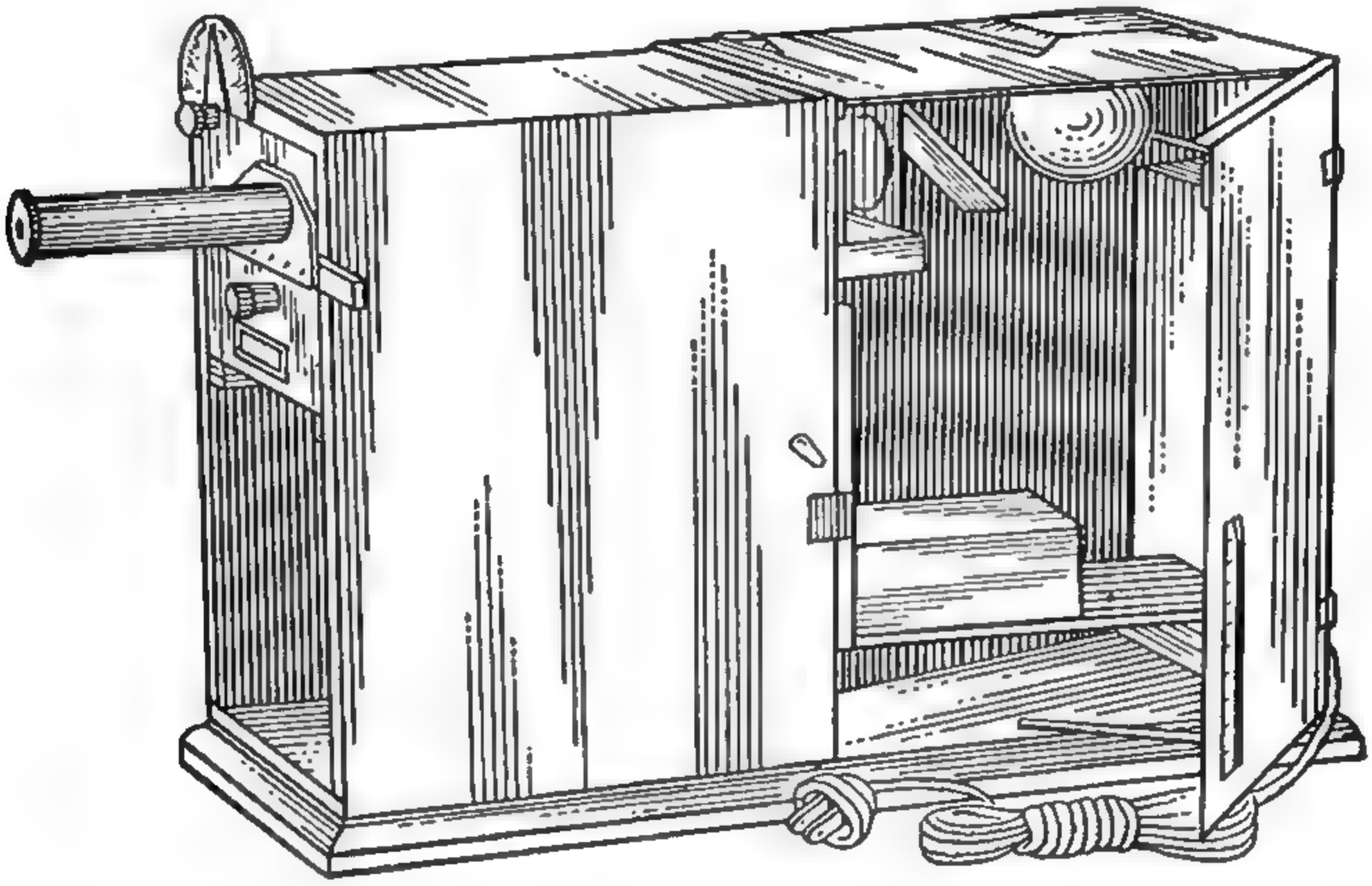
FIG. 248. Photometric device of the Pulfrich photometer.

The instrument may also be set up vertically and used as a colorimeter of the Duboscq type (Fig. 247). Special plunger cells, placed on a stage, are used for this purpose. The lamp house is set at an angle of 45°, and the light is reflected into the colorimeter by means of a mirror. With a similar set-up, reflectance measurements may be made on solid substances by comparison with a white surface. A spherical reflector, providing diffuse reflection, is also furnished for color determinations on solid substances.

Ives Tint-Photometer. The photometric device of this instrument consists of two broad rectangular slits of equal width. One of these can be gradually closed by means of a shutter actuated by a rack and pinion, and the percentage opening is read on a semicircular scale. Several light filters are provided each of which cuts out a certain spectral band: red (625 mμ), yellow-green (575 mμ), green (535 mμ), blue-green (500 mμ), and blue (465 mμ). A screen having an effective wavelength of 560 mμ may also be obtained. Since some of these filters cut rather wide bands out of the spectrum, a good match between the

two halves of the field is often difficult to obtain. To overcome this difficulty the depth and concentration of the sugar solution should be chosen so that the optical center of gravity of the filter coincides as closely as possible with that of the corresponding band in the sample.

The instrument is shown in Fig. 249, and the details of construction may be seen from Fig. 250. The light source is a polished-surface white bulb, mounted in such a position that the two halves of the field are



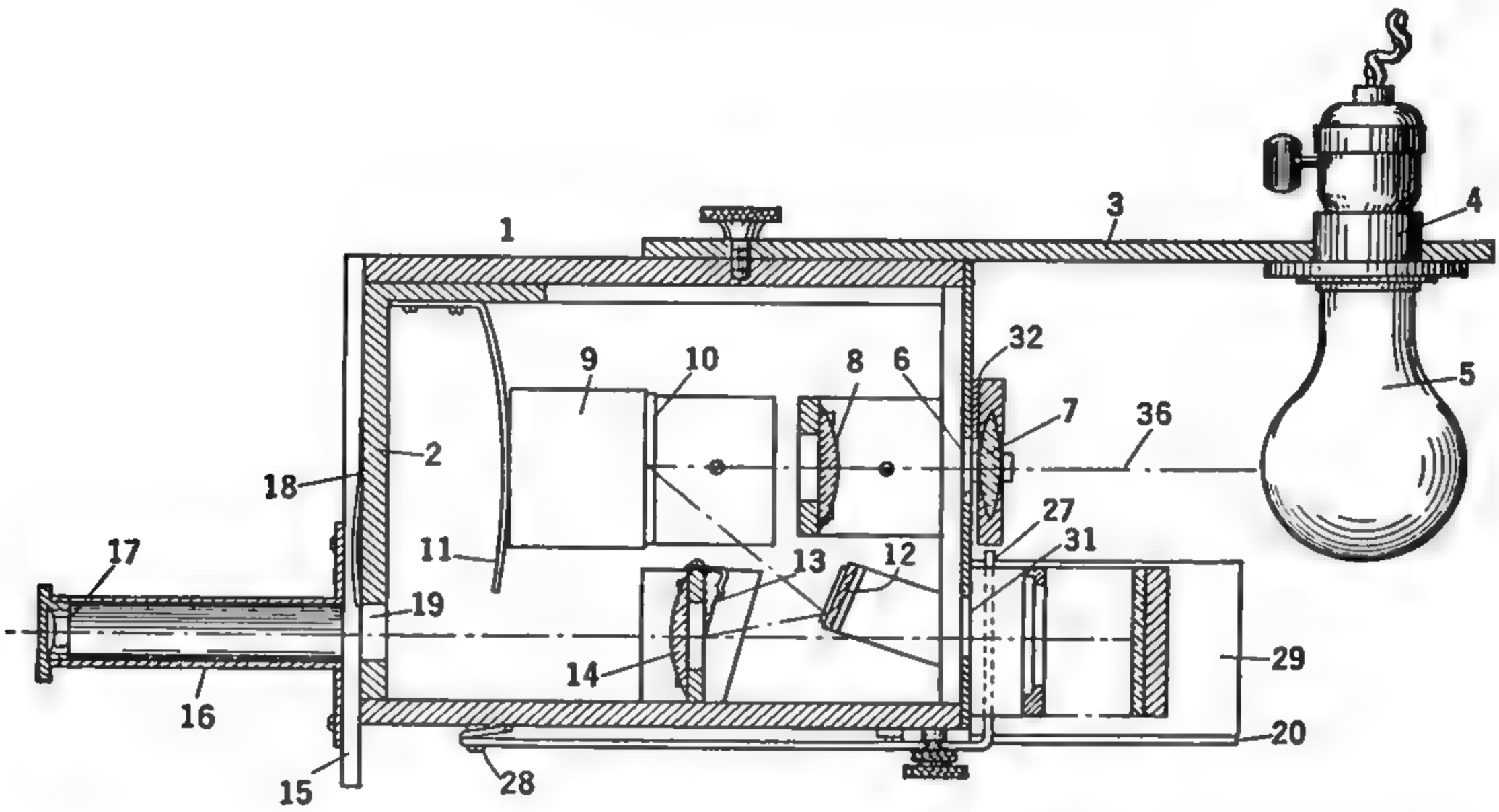
(Courtesy of Palo-Myers, Inc.)

FIG. 249. Ives tint photometer.

evenly illuminated. One light bundle passes through the adjustable slit, through lenses (7) and (8), is reflected by magnesia block (9), again reflected by mirror (12), and passes through prism (13), covering one half of the field of vision, into the eyepiece. The other light bundle is reflected by magnesia block (29), again reflected by a mirror, then passes through the cell for liquid samples, and through the slit of constant width and through lens (14) to the eyepiece, forming the other half of the field of vision.

To measure the transmittancy of a solution, one of the color screens is placed in the slot behind the eyepiece. One of a pair of cells, ranging in thickness from 3 to 150 mm., is filled with distilled water and placed on the shelf behind the slit of constant width. The lever on top of the instrument, regulating the width of the adjustable slit, is set at 100 on the scale, and the magnesia block (29) is raised or lowered until the two halves of the field match in brightness. Then the other cell, of the same thickness, and filled with the solution to be examined, is substituted for the water cell, and the pointer on the scale is moved until a

match is again obtained. The scale reading then indicates the transmittancy, in percentage, for the particular filter used. A logarithmic scale, reading directly in —log T, may be employed, as proposed by Rice.[113] For reflection measurements, the 100 point is adjusted as described, but without interposing an absorption cell; the sample is then placed directly on the magnesia block, which is lowered until the surface of the sample is in the same plane as that of the magnesia block after adjustment of the 100 point. The reading, made as usual, gives



(*Courtesy of Palo-Myers, Inc.*)

FIG. 250. Showing construction of Ives tint photometer.

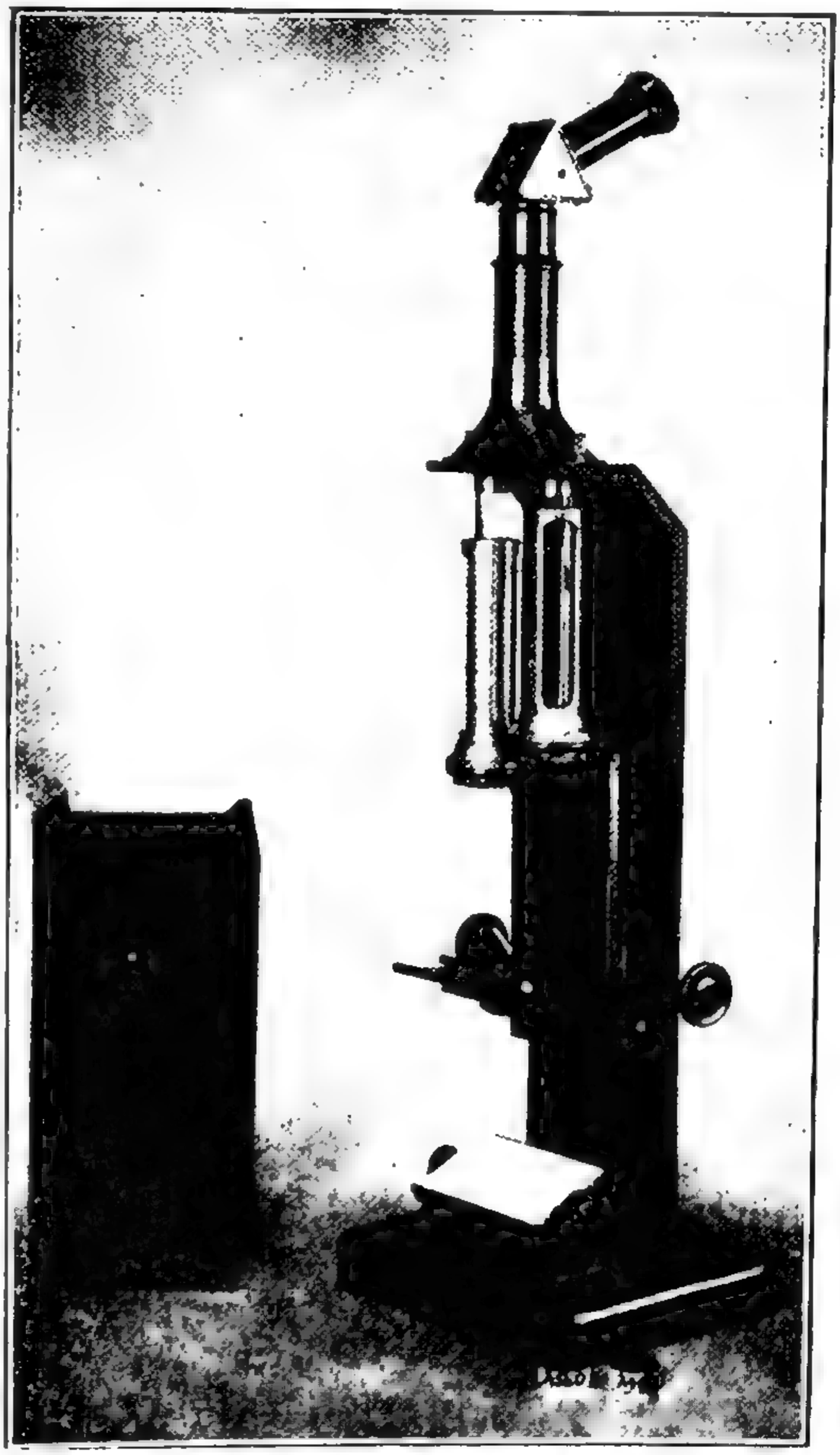
directly the percentage reflectance under the given experimental conditions.

Instruments for determining transmittancy photometrically with the aid of photoelectric cells rather than by visual observation, and equipped with color filters instead of dispersion prisms, have been designed by Šandera,[114] Lange,[115] Holven and Gillett,[116] Keane and Brice,[117] Schmidt and Haensch,[118] Adam Hilger,[119] and a number of others. Some of these apparatus (Keane and Brice, Holven and

[113] *Louisiana Planter*, 73, 392 (1924).
[114] *Z. Zuckerind. čechoslovak. Rep.*, 52, 261 (1927/28); 55, 33 (1930/31).
[115] *Deut. Zuckerind.*, 59, 692 (1934); see also ibid. 379, 397.
[116] *Facts About Sugar*, 30, 169 (1935); *Ind. Eng. Chem.*, 28, 391 (1936).
[117] *Ind. Eng. Chem., Anal. Ed.*, 9, 258 (1937).
[118] Landt and Hirschmüller, *Z. Ver. deut. Zucker-Ind.*, 87, 449 (1937); 88, 247 (1938).
[119] Pamphlet of Adam Hilger, Ltd., on the "Spekker Absorptiometer."

Gillett) may be used also for reflection measurements. Several are equipped with a sodium or mercury lamp as monochromatic light sources.

Brewster's Simplified Color-Measuring Instrument.[120] This apparatus, Fig. 251, is a Duboscq colorimeter, modified to measure transmittancy. Monochromatic light is obtained by inserting in the eyepiece the filter for 560 or 460 mμ, described on p. 596, so that it rests on a diaphragm, or on the lower lens mounting. The height of the solution to be measured is adjusted, as in the Stammer instrument, against a photometric standard glass plate, the transmission of which at 560 and at 460 mμ is known. The standard plate is supported on a brass shelf with a heavy bracket which is rigidly fastened to the vertical column of the colorimeter with two screws. A clearance of 1 cm. is left between the top of the shelf and the lowest position of the cup stages, as shown in Fig. 251. The shelf has two openings, 25 mm. in diameter, and centered directly underneath the cups. A plate carrier with a 25-mm. opening, over which the standard plate is placed, slides between guides on the shelf. A stop at the center of the shelf engages in a slot in the carrier and serves to center the openings under either stage when the carrier is



Reproduced with permission from J. Research Natl. Bur. Standards, 16, 352.)

FIG. 251 Brewster's simplified apparatus for technical sugar colorimetry.

[120] *Bur. Standards J. Research,* 16, 349 (1936).

moved from side to side. Colorimeters of other makes may be fitted similarly, by providing for an easy change of standards from one side to another.

Satisfactory standard plates may be made of carbon amber glass of the American Optical Co., Southbridge, Mass. The transmission curves of these glasses are similar to the curves of caramel, so that a good color match can be obtained with sugar solutions, even when the blue and green filters used have rather wide spectral bands. The transmission of the plates, at 560 and 460 mμ, must be measured with the spectrophotometer.

Because of the slight asymmetry of most colorimeters, and the difficulty in getting a perfect 0 setting, the color measurements are made by a transposition method. A portion of the sugar solution is placed in each of the two colorimetric cups. The standard plate is inserted under one cup, the corresponding scale set at a definite blank reading, say 1 cm., five readings are taken, and the results are averaged. The standard plate is then shifted under the other cup, the corresponding scale set at 1 cm., and another series of readings is taken. The ten results are averaged, and the blank setting is deducted. The difference gives the thickness, b, in the Lambert-Beer equation, for a transmittancy[121] equal to the transmission of the standard plate. Supposing that the green filter was inserted in the eyepiece, that the standard plate has a transmission of 57.9 per cent at 560 mμ, corresponding to a $-\log t$ of 0.2373, that b was found to be 2.18, and the concentration of the solution was 0.7867 g. per ml., then

$$-\log \text{t of the colored dry substance} = \frac{0.2373}{2.18 \times 0.7867}, \text{ or } 0.1384$$

Comparisons by Brewster with spectrophotometric measurements gave very close agreement, except for very pale solutions. The simplified instrument is very well suited for technical sugar color determinations.

Preparation of Sugar Solutions for Color Analysis. If the concentration of coloring matter is to be determined this must be done with a solution of the sugar product because only under this condition can Beer's law be applied. Reflectance measurements on solid sugars are affected not only by the quantity of coloring matter but also by the reflection angle, by the size of the crystals, and by other factors.

It has been found best to prepare solutions of high concentration, at least 60 Brix. With high-grade sugars the concentration may be increased to 65 Brix provided that the subsequent filtration is not slowed

[121] Actually the transmittance, but the absorption of pure water is negligible.

up too much. Dilution with water disturbs the colloid equilibrium and affects the transmittancy of the filtrate. Solid sugars are dissolved by slowly adding the calculated amount of boiling-hot water to a weighed amount of sugar in a tared flask, with constant stirring to keep the solution near the saturation point at all times. A sufficient amount of dry, colorless sucrose is added to juices, etc., below 60 Brix to bring the final concentration to 60 Brix or higher. Very dark products, like molasses, are also diluted with pure, colorless sucrose, as will be described later (p. 606).

Filtration of the Solution. Solutions of sugar products are nearly always more or less turbid, and this turbidity increases the absorption caused by coloring matter. One way to eliminate the effect of turbidity is removal of it by means of an appropriate filtering agent. Peters and Phelps[122] concluded from an investigation of the subject that specially prepared asbestos is the only material that gives satisfactory filtrates for spectrophotometric measurements. But various forms of diatomaceous earth are also widely used. Balch[123] recommends purified Filter-Cel, Zerban and Sattler[124] use Celite Analytical Filter Aid; the Java Sugar Experiment Station[125] prescribes Hyflo Supercel. Spengler and Landt[126] have used quantitative filter paper (three thicknesses of S.S. No. 589, Blue Ribbon) with good success. Lundén[127] advocates centrifuging for 2 hours with 1000 times gravity.

Asbestos Filtration. Brewster and Phelps[128] have simplified the original procedure of Peters and Phelps. The asbestos (Powhatan Mining Co., Grade XXX, XX, or A) is first purified by adding to 25 g. of it 250 ml. of sodium hydroxide solution of sp. gr. 1.284 (20°/4° C.). This mixture is boiled for 30 minutes in a Pyrex flask or a clean vessel of iron or nickel. After digestion the mixture is filtered hot by suction, and washed repeatedly with hot water. The asbestos, which has been pressed in the Büchner funnel to force out most of the water, is transferred to a flask and treated with 250 ml. of concentrated hydrochloric acid and 25 ml. of concentrated nitric acid. The mixture is shaken so that the pulp is disintegrated, and then heated 30 minutes on the water bath. At the end of this period 250 ml. of hot water is mixed with the contents of the flask and the asbestos is filtered by suction as before

[122] *Bur. Standards Tech. Paper* 338, 1927.
[123] *Ind. Eng. Chem., Anal. Ed.*, 3, 124 (1931).
[124] *Ind. Eng. Chem., Anal. Ed.*, 8, 168 (1936).
[125] "Methoden van Onderzoek bij de Java-Suikerindustrie," 6th ed., p. 211, 1931.
[126] *Z. Ver. deut. Zucker-Ind.*, 77, 454 (1927).
[127] *Centr. Zuckerind.*, 36, 575 (1928).
[128] *Ind. Eng. Chem., Anal. Ed.*, 2, 373 (1930); *Bur. Standards J. Research*, 10, 365 (1933).

and washed repeatedly with hot distilled water until all acid is removed. The purified asbestos must be protected from dust. It is dried in an oven at 110° C. and stored in a clean glass container.

Two filters with pads of the purified asbestos are prepared before the sugar solution is made up. Suitable forms of filtering devices are 25-ml. Gooch crucibles with a circular disk of 200-mesh bolting silk in the bottom, or Jena sintered glass funnels, No. 1 porosity for the preliminary filtration, and No. 2 or 3 for the final filtration. The asbestos is shaken up with water and the slurry poured on the filter, sucked down by means of the vacuum pump, and packed tightly by tamping with a blunt stirring rod to produce a layer about 0.5 cm. thick. The pad is then washed several times with water until the fine fibers have been completely removed, as shown by examination of the filtrate in a strong beam of light in the dark room.

During its preparation the solution of the sugar product is kept hot by immersing the flask in a water bath heated to 80–90° C. When solution is complete, purified asbestos (grade A) in amount equal to about 0.5 per cent of the weight of the solution is added. The flask is closed with a clean rubber stopper and vigorously shaken. It is returned to the hot-water bath, and the preliminary filter is heated by rinsing with hot distilled water which is removed as thoroughly as possible by suction. A few milliliters of the sugar solution is poured on the pad and drawn through to displace the water. The solution is now poured on the filter, and after a few drops have filtered the suction is stopped and a clean receiver is substituted. The pads are always kept covered with solution during the filtration, and the suction is stopped before the pad becomes uncovered at the end of the filtration. The receiver is detached and returned to the bath while the final pad is being rinsed with hot water and drained. The second filtration is carried out exactly as the first, but without further addition of asbestos to the first filtrate. The main portion of the second filtrate is again collected in a clean receiver. The bottle is cooled, wiped dry on the outside, and closed with a clean, dry stopper. The contents of the bottle are thoroughly mixed, the refractive index of the solution is determined, and the remainder is used for examination in the spectrophotometer or other color instrument used. If the second filtrate appears turbid the whole operation is repeated with a fresh solution, preferably with increased dry-substance concentration.

The prolonged heating prescribed by Brewster and Phelps during the preparation and filtration of the solutions is a very questionable practice. Although the heating hastens the filtration, it is nevertheless well known that the color of sugar products rapidly increases at high

temperatures through incipient caramelization, reaction of reducing sugars with amino compounds, etc. Hot water is necessary in the preparation of the solutions, but after that the operations should be carried out at room temperature.

Filtration with Hyflo Supercel. In the method of the Java Sugar Experiment Station the Hyflo Supercel is purified by heating 1 kg. with 2 liters of concentrated hydrochloric acid for 3 hours to 80° C. The Hyflo Supercel is then washed with hot water, first by decantation, and finally on a filter. It is dried at 110° C. Two grams is intimately mixed with 200 g. of the sugar solution, and the mixture is filtered by suction through a large glass Gooch crucible, with the aid of a hardened filter paper, S.S. No. 575. Filtration through the same filter bed is repeated five times, and the final filtrate is collected in a clean receiver.

Filtration with Celite Analytical Filter Aid. Zerban and Sattler purify this material further by boiling 75 g. of it with 100 ml. of concentrated hydrochloric acid and 900 ml. of water, filtering hot on a Büchner funnel, and washing thoroughly with hot water. This treatment is repeated three times, and the Celite is finally dried, and ignited in a muffle. In routine work 6 g. of the prepared Celite is well shaken with 100 g. of the sugar solution, and the mixture filtered through a double layer of filter paper, S.S. No. 589, Blue Ribbon, in a 7-cm. Büchner funnel or large Gooch crucible, with the filtering arrangement shown in Fig. 252. At the beginning of the vacuum filtration the stopcock is left open until about 25 ml. of the liquid has passed through. When the solution runs perfectly clear, the stopcock is closed, and the final, clear portion of the filtrate is collected in the tube above the stopcock, without breaking the vacuum at any time.

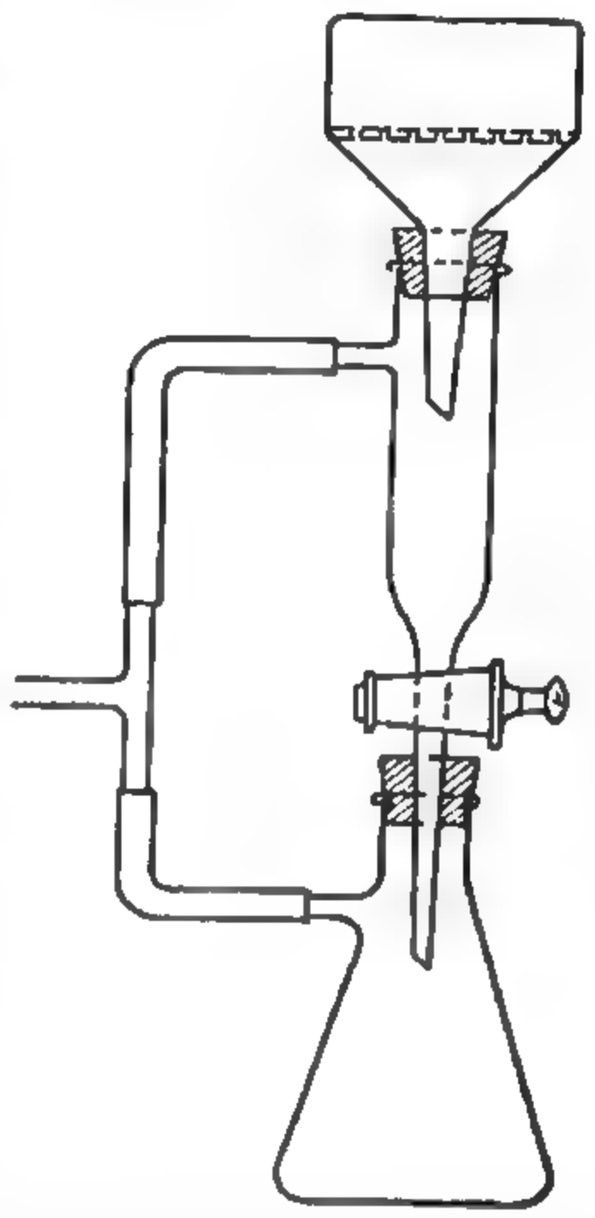


FIG. 252. Apparatus for filtration with Filter-Cel for color determination.

Comparison of Filtering Agents. The choice of filtering agent in the preparation of sugar solutions for colorimetric analysis has long been a matter of controversy. Brewster and Phelps object to the use of any kind of diatomaceous earth, claiming that it not only decolorizes the solutions but also has a selective effect in different parts of the spectrum. Balch, Honig, and others consider diatomaceous earth to be satisfactory. Zerban and Sattler have made a special study of this subject, comparing purified asbestos, purified Celite Analytical Filter Aid, and also specially prepared, finally divided silica gel. All three

filtering agents remove turbidity and coloring matter selectively, depending on the particle size, and the filtrates obtained with any of them always show measurable turbidity. Color and turbidity can therefore not be sharply separated by any of the three, and the choice between them thus becomes a matter of personal preference or mutual agreement. Asbestos has the decided disadvantage that its effectiveness in removing turbidity depends entirely on the way in which the asbestos is packed in the crucible. Tight packing gives lower color values because of ultrafiltration effects. Agreement between the results of different laboratories is therefore difficult to achieve when asbestos is used. Celite usually gives lower color values than asbestos, and silica gel still lower ones, but there are exceptions to this rule as would be expected from the selective effect of all of them.

Determination of the Specific Absorptive Index. A part of the solution, prepared and filtered as described, is used for determining the percentage of total solids, most conveniently by means of the refractometer. The result, multiplied by the corresponding density, and divided by 100, gives the grams of total solids per milliliter of solution, designated by *c* in the formula of Lambert-Beer (p. 591). The transmittancy **T** of the solution is measured as shown on p. 590, with any of the spectrophotometers or other photometric instruments described previously. The optical surfaces of the absorption cells must be free from scratches, scrupulously clean, and must not be touched with the fingers. The white sugar sirup placed in the comparison cell is prepared by mixing a 60 Brix solution of the best grade of granulated sugar with 2 per cent (on the weight of dry sugar) of a good decolorizing carbon, heating the mixture to near the boiling point, filtering through paper on a Büchner funnel, and finally through purified asbestos or Celite. The effective thickness of the absorption cells, in centimeters, represents *b* in the formula of Lambert-Beer.

The —log **t**, computed from *b*, *c*, and —log **T** at wavelength 560 mμ, is a measure of the concentration of coloring matter in the sample, and may be converted, if desired, into Peters and Phelps' units of coloring matter by dividing by 0.00485.

Treatment of Dark-Colored Products. If the sugar product to be examined is very dark, either very thin absorption cells may be used, down to 1 mm. or less, or else the color may be diluted. As has been mentioned before, water must never be used for this purpose. The product is accurately weighed into a volumetric flask and diluted to the mark with white sugar sirup, prepared as described above. This sirup does not keep well, however, and according to Brewster and Phelps it is preferable to dilute with solid sucrose and water. One

hundred grams of highest-grade refinery tablets is weighed into a tared flask. A small amount of the colored sample whose content of total solids must be known is added, and the flask is reweighed. The mixture is dissolved by slowly adding boiling hot water with constant stirring; the final concentration should be 60 to 65 Brix. The transmittancy of this solution is determined as usual, but the concentration c of the colored dry substance must be calculated from the weight or volume and the Brix of the colored product, the weight or volume and Brix of the colorless sirup, and the Brix of the mixture before and after filtration.[129] In work of high precision the $-\log T$ obtained for the mixture, especially when measurements are made at the blue end of the spectrum, must be corrected for the slight absorption caused by the white sucrose. This is done by subtracting from the observed $-\log T$ of the mixture at any wavelength the $-\log T$ of the sugar sirup at the same wavelength, reduced to the basis of its partial concentration in a mixture.

Effect of Reaction on Color. Zerban[130] has shown by a spectrophotometric study of raw cane sugar that an increase in the pH of the solution between the limits of pH 5.8 and 8.3 causes a shifting of the entire absorption curve in the same way as if more coloring matter of the same type had been added. These investigations were extended by Lundén,[131] who disclosed the presence of several coloring matters in beet and cane products, which react differently to changes in pH. Caramel color is characterized by a very steep absorption curve, with high Q-ratios at the blue end. The absorption increases continuously with pH. The so-called "old" color has an absorption curve similar to that of caramel color, but the intensity of absorption is independent of the pH over a wide range. The so-called amethyst color found in some sugars has a flatter absorption curve, and the absorption goes through a maximum at pH 6 to 7; this maximum is most pronounced in the yellow region of the spectrum.

It is therefore necessary, in measuring the color of sugar products, to pay close attention to the pH. If direct comparisons are desired a definite pH must be decided upon and maintained throughout. If more complete information on the types of coloring matter present is needed, measurements must be made throughout an extended pH range.

Color Evaluation without Filtration of the Solution. In order to save the time necessary for the filtration of the solutions prior to color

[129] For details of methods of calculation see Peters and Phelps, *Bur. Standards Tech. Paper* 338, pp. 285–289, 1927.

[130] *Intern. Sugar J.*, 27, 446 (1925).

[131] *Z. Ver. deut. Zucker-Ind.*, 76, 780 (1926).

determination, Keane and Brice[132] have suggested a rapid proximate method for grading white sugars, with the use of unfiltered solutions. A 60-Brix solution of the sugar is prepared, and the transmittancy of the solution is determined in a 15-cm. cell with white light filtered through a blue-green filter (Corning light shade blue-green No. 428, 3.4 mm. thick, effective wavelength 535 $m\mu$), and also through a red filter (Corning traffic red No. 245, effective wavelength 655 $m\mu$). Designating the transmittancies found by T_g and T_r, respectively, the "apparent color index" is calculated by the expression $100\ (1 - T_g/T_r)$. This formula is based on the observation that the color increases as the ratio of T_g to T_r decreases, and on the assumption that the turbidity present in the solution does not affect this ratio. In reality, this assumption is not correct, and there is no straight-line relationship between the concentration of coloring matter and the apparent color index. Keane and Brice, who used a photoelectric photometer of their own design in this work, recommend the method only as a rapid procedure for industrial control.

Nees,[133] using the photoelectric photometer of Lange and various color filters, has measured the absorbency (1 — transmittancy) of solutions of beet granulated sugars, varying the color concentration at constant turbidity, and vice versa. The solutions contained 50 g. of sugar in 100 ml., and were read in a 34-mm. cell. The absorbency due to coloring matter alone, for the blue filter of the Lange apparatus, was found to average 3.1 times that for the yellow filter. The absorbency due to turbidity alone, for the blue filter, averaged 1.05 times that for the yellow filter. Assuming that at one and the same wavelength the absorbency caused by very small quantities of coloring matter and turbidity is directly proportional to their concentration, the following formulas were set up:

$$x + y = a$$

$$\frac{x}{3.1} + \frac{y}{1.05} = b$$

where a is the total absorbency for the blue filter, b the same for the yellow filter, x the absorbency due to color alone for the blue filter, and y that due to turbidity alone for the blue filter. Solving for x and y,

$$y = \frac{b - 0.323\,a}{0.63}$$

[132] *Ind. Eng. Chem., Anal. Ed.*, **9**, 258 (1937).
[133] *Ind. Eng. Chem., Anal. Ed.*, **11**, 142 (1939).

or more simply,

$$y = \frac{3.1\,b - a}{2.0}$$

$$x = a - y$$

This method assumes that the ratios between the absorbencies at the two wavelengths used are constant, for color as well as for turbidity, and that the absorbency is an additive property. Actually, these assumptions are only approximately correct and are applicable only to products which show little variation in color and turbidity, such as white granulated sugars.

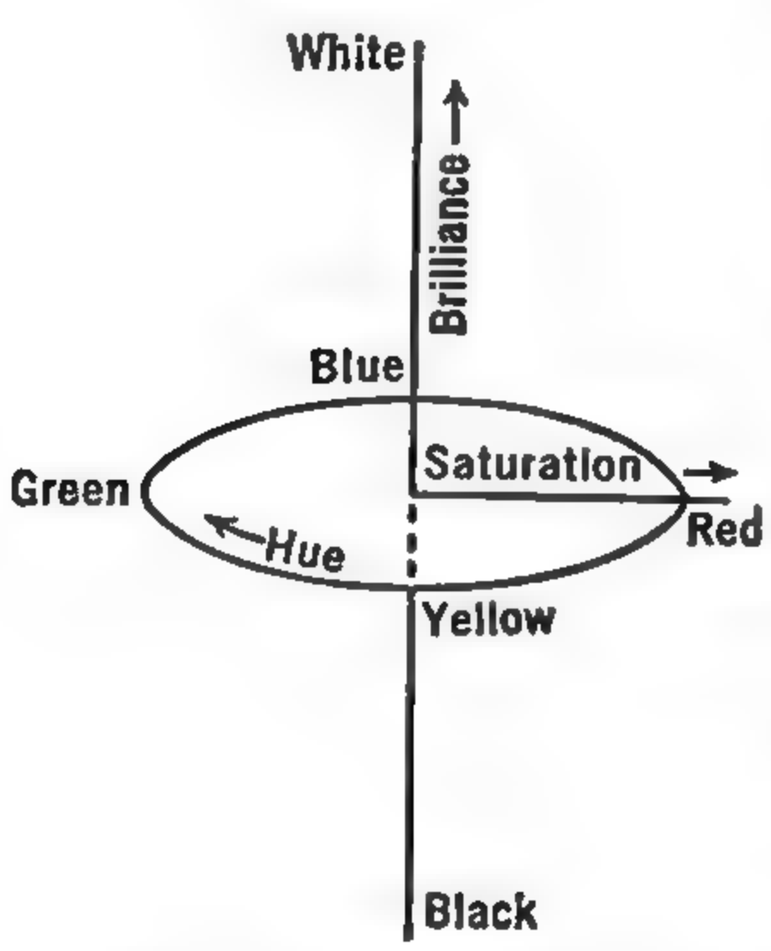
The color and turbidity units used by Keane and Brice and by Nees are entirely arbitrary. Their methods should be used only for the purpose for which they were devised, and their limitations should always be kept in mind.

The method of Zerban, Sattler, and Lorge, for determining color and turbidity, without having recourse to filtration, is described on pp. 633 to 640.

Reflection Measurements on Solid Sugars. While transmittancy determinations on colored sugar solutions are usually made for the purpose of determining the concentration of coloring matter, solid sugars are measured by reflection in order to specify the color as it is perceived by the human eye. This color sensation varies not only with the nature and quantity of the coloring matter present, but also with the physical structure of the sugar and its gloss, with the angle of reflection, and with the nature, intensity, and position of the light source. It is therefore necessary to specify these conditions and to adhere to them if comparable results are to be obtained. For routine purposes it is often sufficient to measure the percentage reflection at one or more wavelengths under the experimental conditions provided by the particular instrument used, some of which have been described. If a spectrophotometer is available the entire reflection curve may be plotted and used for comparisons. This system is helpful in the preparation of standard sugar samples, especially of soft sugars, so that they may be readily duplicated. Judd and Gibson[134] have shown that in such measurements the sample and the white standard must not be covered with a glass because errors of as much as 10 per cent may arise when two surfaces, one light and the other dark, are compared. This restricts reflection measurements upon sugars to instruments in which the surfaces of the sample and standard are placed horizontally.

[134] *Bur. Standards J. Research*, **16**, 261 (1936).

Color Attributes. If it is desired to express numerically the color of a sugar as it appears to the eye, use is made of the attributes of color in the psychological sense. The three attributes by which any color may be characterized,[135] are brilliance, hue, and saturation. They are shown graphically in a three-coordinate system, Fig. 253. Brilliance, indicated by the vertical line, is that attribute by which all colors are arranged in a series from white through various shades of gray to black. A percentage scale on which 100 denotes white and 0 black is generally employed. The physical equivalent of brilliance is called brightness. The hue differentiates a color from a gray of the same brilliance by classing it as reddish, yellowish, greenish, bluish, etc. In the graph the hues are arranged in a circular plane around the vertical axis. Hue is characterized physically by the dominant wavelength. The third attribute, saturation, determines the degree of difference, for a color possessing a hue, from a gray of the same brilliance; it is determined by the ratio of homogeneous to total light, and is usually expressed in percentage of the latter. The physical equivalent of saturation is termed purity. In the graph the saturation is indicated by the radius of the plane circle, with the 0 point at the center and the 100 point at the periphery.



(Reproduced with permission from J. Optical Soc. Am., 6, 527.)

FIG. 253. Diagram showing color attributes.

Instruments by which the color of an object may be measured either in terms of the proportions of three primary colors (trichromatic analysis), or in terms of brilliance, hue, and saturation (monochromatic analysis) are properly called "colorimeters," in contradistinction to the instruments known by that name to the chemist. There are a number of such instruments, but they have the disadvantage that the results obtained with them depend on color vision, which varies from one observer to another. It is therefore preferable to measure the reflection or transmission curve with the spectrophotometer, and to translate the results into the trichromatic or monochromatic specification, on the basis of the best data available for normal color vision and for a light source of given characteristics. Prior to 1931 the system adopted in 1922 by the Optical Society of America[136] was generally used for this

[135] Troland, *J. Optical Soc. Am.*, **6**, 527 (1922).
[136] *J. Optical Soc. Am.*, **6**, 527 (1922); Ives, *J. Franklin Inst.*, **195**, 23 (1923).

purpose, but in 1931 the International Commission on Illumination[137] introduced a perfected system, largely based on more recent investigations.

The Java Sugar Experiment Station[138] has applied this method to the determination of the color of solid sugars by reflected light, using at first the older procedure, but changing to the international system in 1938. This method can be only briefly described here; for the details the chemist is referred to the original literature.[139]

Spectral Reflectometer. The spectral reflectometer of the Bureau of Standards,[140] shown schematically in Fig. 254, is used for the measurements. The normal brightness of a sample of sugar, relative to that of a standard white surface, is measured throughout the visible spec-

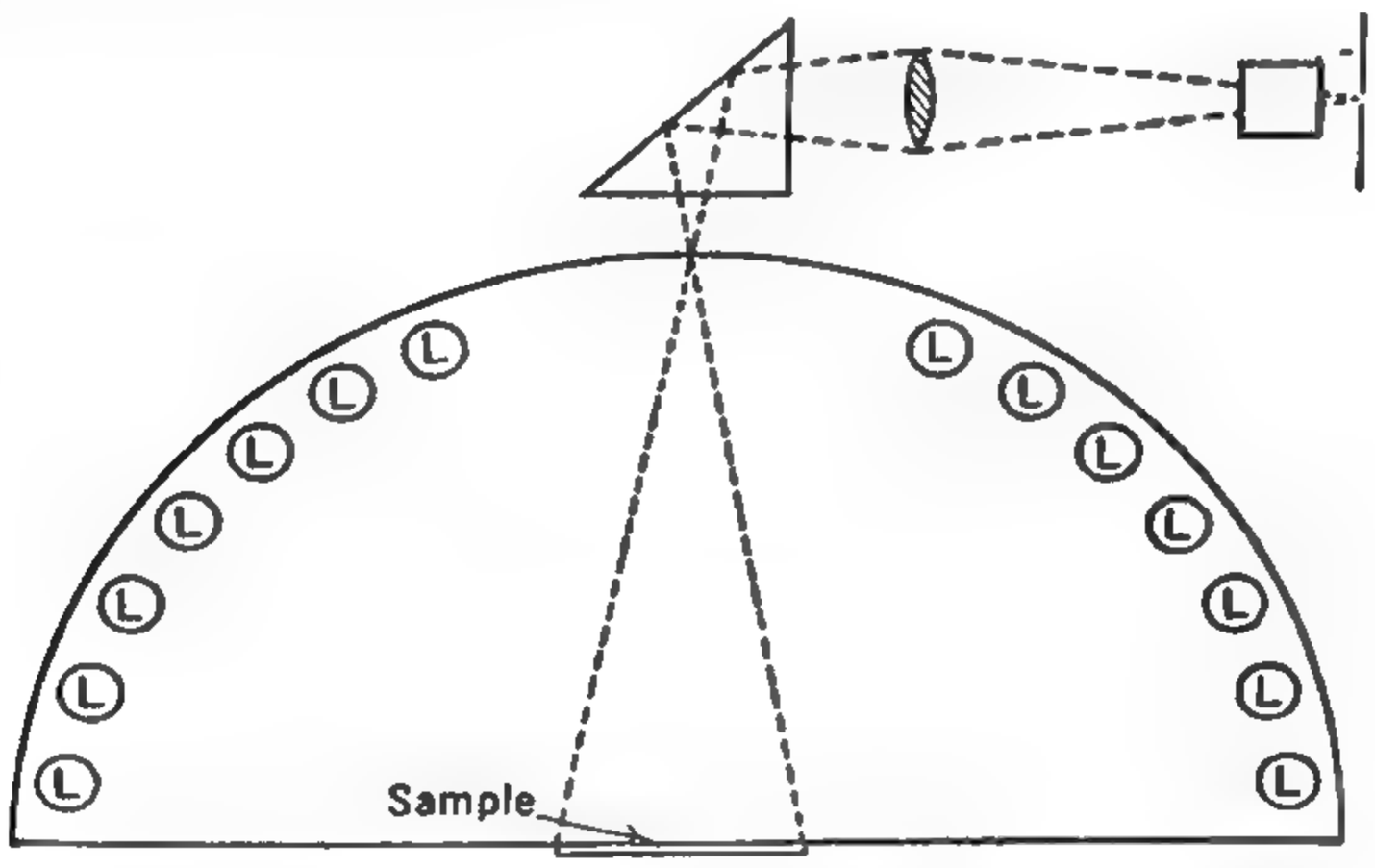


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FIG. 254. Diagram of spectral reflectometer.

trum, at an angle of reflection of 90°, both sample and standard being under equal and perfectly diffuse illumination. This is provided by 156 small electric lamps mounted symmetrically on the inside of a hemisphere. The light of these lamps is reflected by the inner wall which is coated with magnesium oxide. The sample and the standard, having rectangular surfaces of the same dimensions, are placed side by side in the base of the hemisphere so that the dividing line between them coincides with the central dividing line of the base of the hemisphere. The position of the samples is thus eccentric but symmetrical. The sugar sample is placed in a silver dish, 3.5 cm. long, 2.5 cm. wide,

[137] *Trans. Optical Soc.* (*London*), 32, 1 (1930/31); 33, 73 (1931/32).
[138] Honig and Thomson, *Arch. Suikerind.*, 38, III, 1447 (1930).
[139] Hardy, "Handbook of Colorimetry," 1936. Reproduction of tables and graphs by permission of the Technology Press, Cambridge, Mass.
[140] McNicholas, *Bur. Standards J. Research*, 1, 793 (1928).

and 3.9 cm. deep. The standard is a silver plate, thickly coated with magnesium oxide, of the same length and width as the sugar dish. Its surface must be in the same plane as that of the sample. The magnesium oxide coat must be frequently renewed. A white porcelain plate, which can be easily washed, may be used as a working standard, its reflection value being checked from time to time against magnesium oxide. The beams reflected by the sample and the standard pass through diaphragms in the top center of the hemisphere and are again reflected side by side through a system of prisms and lenses into the slits of a spectrophotometer. During the readings the interior of the hemisphere is cooled by a current of air. Readings are taken at a number of points throughout the visible spectrum, and the results are expressed in percentage reflection relative to the magnesium oxide which is called 100. The reflection values are plotted against the wavelengths, and the resulting curve forms the basis for the subsequent calculations.

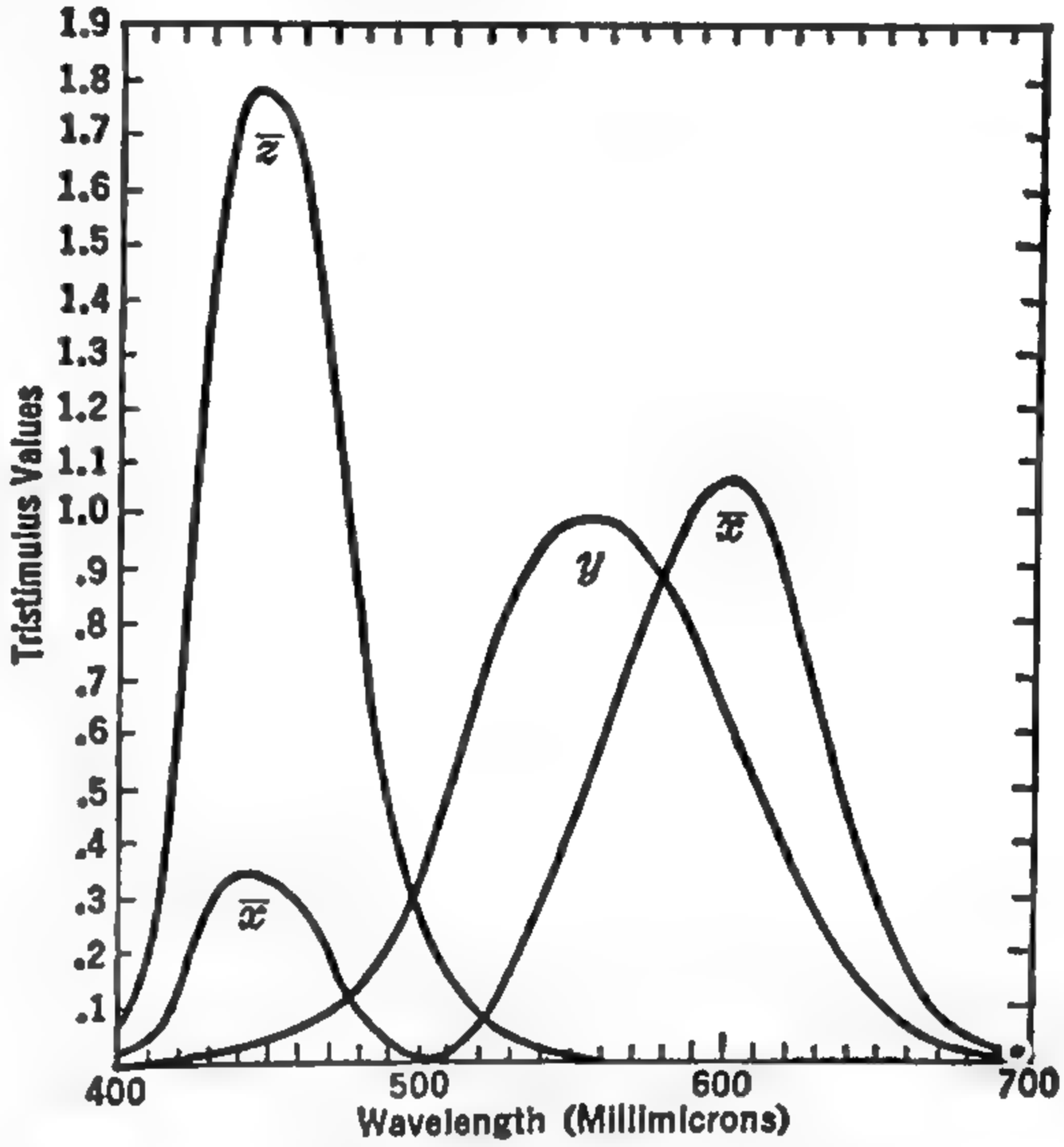
Standard Light Source. Since the color sensation evoked by a material varies with the character of the light by which it is viewed, a standard light source had to be selected, and of the three illuminants adopted by the International Commission on Illumination, designated as A, B, and C, the last named was chosen because it approaches average daylight most closely and therefore best duplicates the conditions under which sugar samples are usually viewed in practice. It is approximately equivalent to a black body at 6500° Kelvin. The relative spectral energy distribution of this illuminant is shown in Table LXXXIII, the energy emitted by illuminant A at wavelength 560 mμ being taken as 100.

TABLE LXXXIII

RELATIVE ENERGY DISTRIBUTION OF ILLUMINANT C

Wavelength	Relative Energy	Wavelength	Relative Energy
400	63.3	560	105.3
410	80.6	570	102.3
420	98.1	580	97.8
430	112.4	590	93.2
440	121.5	600	89.7
450	124.0	610	88.4
460	123.1	620	88.1
470	123.8	630	88.0
480	123.9	640	87.8
490	120.7	650	88.2
500	112.1	660	87.9
510	102.3	670	86.3
520	96.9	680	84.0
530	98.0	690	80.2
540	102.1	700	76.3
550	105.2	...	.....

Tristimulus Values. The first step in the conversion from spectrophotometric to colorimetric data is to find the proportions of the three primaries, red, green, and blue, the combination of which evokes the same color sensation as the sample when viewed by illuminant C. The figures indicating the amount of each of the three primaries that match equal amounts of energy for each pure spectral color (wavelength) are called the "tristimulus" or "excitation" values. They are expressed in arbitrary units. Any desired primaries may be used, and the results obtained with any set of them can be converted by computation into those for any other set. The International Commission on Illumination has recommended the primaries the tristimulus values for which are shown in Fig. 255. This set has the great advantage that the curve for the green primary coincides with the visibility curve for the standard observer, which indicates the relative brightness of the various spectrum colors on an equal energy basis. Hence the tristimulus value of a sample for the green primary gives directly the relative brightness of the sample, expressed as the percentage of a perfect white. The tristimulus values shown in Fig. 255 are given in numerical form in Table LXXXIV, in wavelength steps of 10 mμ.



(Reproduced with permission from Hardy, "Handbook of Colorimetry," p. 8.)

FIG. 255. Tristimulus values of the three primary colors, International Commission for Illumination. *x*, red primary; *y*, green primary; *z*, blue primary.

The tristimulus values for the sample are found by a simplified method of integration. The results of the reflection measurements, (R), expressed as fractions of unity, are multiplied, for each of the wavelengths, 400, 410, 420, etc., to 700 mμ, by the relative energy (E_c) of illuminant C for the same wavelength (Table LXXXIII), and the products are again multiplied by the tristimulus values of the spectrum colors, separately for each of the three primaries, *r*, *g*, and *b*,

respectively (Table LXXXIV). The products are added up, separately for each primary, and the sums obtained give the tristimulus values for the sample.

TABLE LXXXIV

TRISTIMULUS VALUES OF THE SPECTRUM COLORS

Wave-length	*r* Red	*g* Green	*b* Blue	Wave-length	*r* Red	*g* Green	*b* Blue
400	0.0143	0.0004	0.0679	560	0.5945	0.9950	0.0039
410	0.0435	0.0012	0.2074	570	0.7621	0.9520	0.0021
420	0.1344	0.0040	0.6456	580	0.9163	0.8700	0.0017
430	0.2839	0.0116	1.3856	590	1.0263	0.7570	0.0011
440	0.3483	0.0230	1.7471	600	1.0622	0.6310	0.0008
450	0.3362	0.0380	1.7721	610	1.0026	0.5030	0.0003
460	0.2908	0.0600	1.6692	620	0.8544	0.3810	0.0002
470	0.1954	0.0910	1.2876	630	0.6424	0.2650	0.0000
480	0.0956	0.1390	0.8130	640	0.4479	0.1750	0.0000
490	0.0320	0.2080	0.4652	650	0.2835	0.1070	0.0000
500	0.0049	0.3230	0.2720	660	0.1649	0.0610	0.0000
510	0.0093	0.5030	0.1582	670	0.0874	0.0320	0.0000
520	0.0633	0.7100	0.0782	680	0.0468	0.0170	0.0000
530	0.1655	0.8620	0.0422	690	0.0227	0.0082	0.0000
540	0.2904	0.9540	0.0203	700	0.0114	0.0041	0.0000
550	0.4334	0.9950	0.0087	...	.....	.....	.....

The calculation is simplified by forming once and for all the products of the energy values and the tristimulus values of the spectral colors. These products are found in columns 3, 5, and 7 of Table LXXXV.

Trichromatic Coefficients. Calculation of Brightness. The three tristimulus values of the sample are added up together, and each of them expressed as a fraction of the total. The figures thus obtained are called the "trichromatic coefficients." The tristimulus value of the sample for the green primary is divided by the sum of the products in column 5 (brightness = 100 per cent), and the quotient, expressed as per cent, gives directly the brightness of the sample (see p. 613).

The method of calculation is illustrated by a sample of a white sugar measured at the Java Sugar Experiment Station, as shown in Table LXXXV. The reflection values, *R*, obtained by spectrophotometric measurement are given in column 2 of the table. These are multiplied, for each wavelength shown in column 1, by the corresponding figures in columns 3, 5, and 7, respectively, and the results entered in columns 4, 6, and 8, respectively. All the products in column 4 are added up, likewise those in columns 6 and 8. Then the sums for the three columns are added, and each sum, for red, green, and blue, respectively, is divided by the total of the three.

TABLE LXXXV

Calculation of Trichromatic Coefficients and Brightness of a Sample of White Sugar

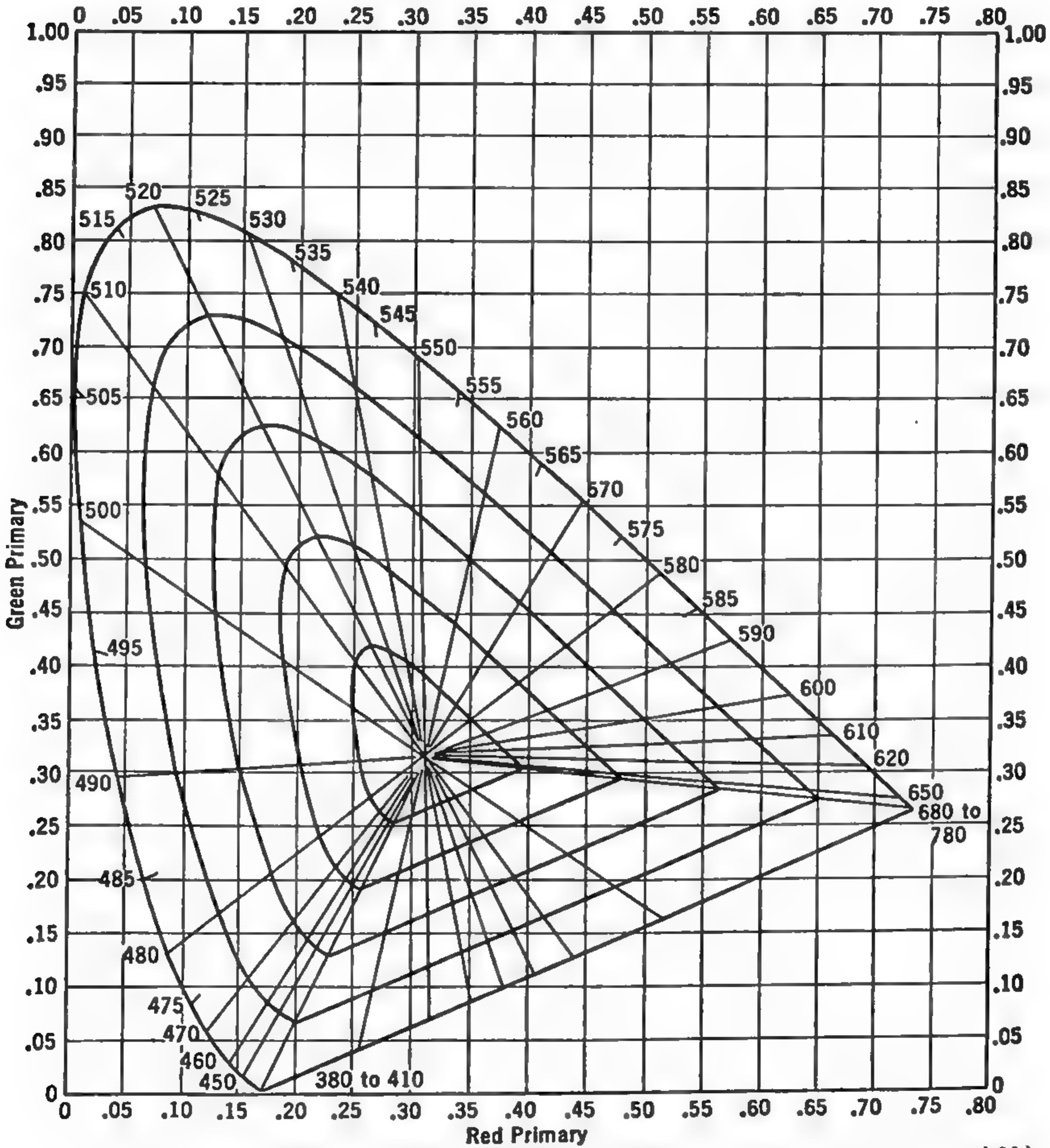
1 Wave-length	2 R	3 E_{cr}	4 RE_{cr}	5 E_{cg}	6 RE_{cg}	7 E_{cb}	8 RE_{cb}
400	0.540	0.91	0.49	0.03	0.02	4.34	2.34
410	0.600	3.48	2.09	0.10	0.06	16.58	9.95
420	0.652	13.19	8.60	0.39	0.25	63.36	41.31
430	0.700	31.92	22.34	1.30	0.91	155.74	109.02
440	0.740	42.15	31.19	2.79	2.06	211.43	156.46
450	0.778	41.69	32.43	4.71	3.66	219.81	171.01
460	0.807	35.81	28.90	7.39	5.96	205.49	165.83
470	0.831	24.19	20.10	11.27	9.37	159.46	132.51
480	0.850	11.85	10.07	17.22	14.64	100.71	85.60
490	0.866	3.86	3.34	25.11	21.75	56.13	48.61
500	0.879	0.55	0.48	36.21	31.83	30.49	26.80
510	0.888	0.95	0.84	51.46	45.70	16.18	14.37
520	0.894	6.13	5.48	68.80	61.51	7 58	6.78
530	0.898	16.22	14.57	84.48	75.86	4.13	3.71
540	0.901	29.65	26.71	97.40	87.76	2.08	1.87
550	0.904	45.60	41.22	104.67	94.62	0.92	0.83
560	0.907	62.60	56.78	104.77	95.03	0.41	0.37
570	0.909	77.97	70.88	97.39	88.53	0.21	0.19
580	0.911	89.61	81.64	85.09	77.52	0 15	0.14
590	0.914	95.68	87.45	70.55	64.48	0.11	0.10
600	0.916	95.26	87.26	56.60	51.85	0.07	0.06
610	0.920	88.65	81.56	44.47	40 91	0 03	0.03
620	0.923	75.28	69.48	33 57	30.99	0.02	0.02
630	0.927	56.54	52 41	23.32	21.62	0 01	0.01
640	0.931	39.32	36 61	15.37	14.31	0 00	0.00
650	0.935	25.01	23.38	9.44	8 83	0.00	0.00
660	0.940	14.50	13 63	5.36	5.04	0.00	0.00
670	0.945	7.54	7.13	2 76	2 61	0 00	0.00
680	0.952	3.93	3.74	1 43	1 36	0.00	0.00
690	0.958	1.82	1.74	0.66	0 63	0 00	0.00
700	0.965	0.87	0.84	0 31	0 30	0 00	0.00
Sums			923.38	1064 42	959.97		977.92

Sum of $R \times Ec \times r$ = 923.38
Sum of $R \times Ec \times g$ = 959.97
Sum of $R \times Ec \times b$ = 977.92
Total 2861.27

Trichromatic coefficient for r: 923.38 ÷ 2861.27 = 0.3227
Trichromatic coefficient for g: 959.97 ÷ 2861.27 = 0.3355
Trichromatic coefficient for b: 977.92 ÷ 2861.27 = 0.3418
1.0000

Brightness: 100 × 959.97 ÷ 1064.42 = 90.2

Graphic Determination of Dominant Wavelength and Purity. The dominant wavelength and purity corresponding to the trichromatic coefficients are found from a chromaticity diagram or, as it is usually called, a color diagram. Such a diagram, for illuminant C and for the



(Reproduced with permission from Hardy, "Handbook of Colorimetry," pp. 61 and 86.)

FIG. 256. Color diagram for converting trichromatic coefficients into dominant wavelength and purity.

primaries and the standard observer of the International Commission on Illumination, is shown in Fig. 256. Since the sum of the trichromatic coefficients is always equal to 1, the color of the sample is fully defined by only two of them, and this makes it possible to use rectangular

coordinates instead of a triangle. It is common practice to plot the trichromatic coefficients for the red primary as abscissas, and those for the green primary as ordinates. The spectrum colors (purity = 100 per cent) are all located on the outer contour curve. The location of illuminant C is at the intersection of its trichromatic coefficient for the red primary, 0.3101, and of that for the green primary, 0.3163; at this point the purity equals 0. All the colors on a radial line connecting this point with the outer contour curve have the same dominant wavelength. The four equidistant concentric curves between the point indicating illuminant C and the outer contour curve indicate intermediate purities of 20, 40, 60, and 80 per cent, respectively. It is thus possible to interpolate for any color both the dominant wavelength and the purity. In practice a greatly enlarged graph must be used for precise interpolation.[141]

Taking again the white sugar, the calculations for which have been given in the example above, the intersection of the trichromatic coefficient for the red primary, 0.3227, and of that for the green primary, 0.3355, is found to lie on the radial line indicating wavelength 572 mμ, and this is the dominant wavelength of the sample. The purity is found to lie between the 0 point and the concentric line indicating 20 per cent purity; interpolation gives 8.6 per cent purity for the sample. This purity is called the " excitation " purity. It may be converted into the " colorimetric " purity by multiplying by the trichromatic coefficient for the green primary, at wavelength 572 mμ and 100 per cent purity (in the example 0.5408), and dividing the product by the trichromatic coefficient of the sample for the green primary (0.3355). The result is $8.6 \times 0.5408 \div 0.3355 = 13.9$. The final result for the white sugar sample may be summarized as follows:

Trichromatic analysis: trichromatic coefficient for the red primary, 0.3227; for the green primary, 0.3355; for the blue primary, 0.3418.

Monochromatic analysis: brightness, 90.2 per cent; dominant wavelength, 572 mμ; excitation purity, 8.6 per cent; colorimetric purity, 13.9 per cent.

In the color diagram, Fig. 256, there is also a straight line connecting the extreme blue with the extreme red in the outer contour curve; this line is used for specifying purple colors by means of their complementary colors. This part of the curve need not be considered in determining the color of sugars.

Calculation of Dominant Wavelength and Purity. The graph, Fig. 256, is based on tables, and these same tables may be used for a

[141] Such a graph, in sections, is to be found in Hardy's " Handbook of Colorimetry," and can be purchased separately.

direct calculation of the dominant wavelength and purity. In order to do this, the tristimulus values of the spectral colors, given in abridged form in Table LXXXIV, are converted, wavelength for wavelength, into the trichromatic coefficients, as explained previously (p. 614), and a table of trichromatic coefficients for the spectral colors is prepared.[142] Then the trichromatic coefficient of the red primary for 0 purity (0.3101) is, for each wavelength, deducted from the trichromatic coefficient of the red primary for 100 per cent purity. Similarly, the trichromatic coefficient of the green primary at 0 purity (0.3163) is deducted from that of the green primary at 100 per cent purity. The difference for the red primary is, for each wavelength, divided by the difference for the green primary, and a table is constructed showing these ratios in steps of 1 mμ throughout the visible spectrum. This ratio table in turn serves to locate the dominant wavelength of a sample from the corresponding ratio found for the sample. Taking again the white sugar, Table LXXXV, as an example, the difference between the trichromatic coefficient of the sample and that at 0 purity, for the red primary, is $0.3227 - 0.3101 = 0.0126$; that for the green primary is $0.3355 - 0.3163 = 0.0192$. The ratio between the two differences is $0.0126 \div 0.0192 = 0.6562$. The ratio table shows for wavelength 571 mμ a ratio of $(0.4511 - 0.3101) \div (0.5478 - 0.3163) = 0.6091$, and for wavelength 572 mμ a ratio of $(0.4581 - 0.3101) \div (0.5408 - 0.3163) = 0.6592$. The ratio found for the sample, 0.6562, corresponds, by interpolation, to dominant wavelength 571.9 mμ, against 572 mμ found from the graph.

The excitation purity of the sample is calculated in a similar manner. The trichromatic coefficient of the red primary for the spectral color (100 per cent purity) 571.9 mμ is 0.4577, that at 0 purity is 0.3101, and that for the sample was found to be 0.3227. The percentage purity is therefore $100 \times (0.3227 - 0.3101) \div (0.4577 - 0.3101)$, or 8.54 per cent, against 8.6 per cent found from the graph. If the same calculation is made for the green primary, the result is $100 \times (0.3355 - 0.3163) \div (0.5412 - 0.3163)$, which also equals 8.54.

The method of calculation described is known as the " weighted ordinate " method. Though intrinsically simple, it is nevertheless very laborious and time-consuming. For samples which, like many sugars, have a smooth reflection curve it is usually possible to reduce the number of wavelengths measured and used for the calculation to every 20, 30, or even 40 mμ, without material loss in accuracy.

Selective Ordinate Method of Hardy. Still more time may be saved by using the computation procedure known as the " selective ordinate "

[142] Such a table is found in Hardy's " Handbook of Colorimetry," p. 55.

method originated by Hardy. In this method it is not the wavelengths that are equally spaced, but rather the products of the relative energy of the light source and the tristimulus values. Any desired number of ordinates throughout the visible spectrum may be selected. Hardy has calculated tables for 100, 30, and 10 ordinates, respectively. In the case of sugars 10 ordinates are usually sufficient. The corresponding wavelengths are naturally different for the three primaries. The wavelengths computed by Hardy for the 10-ordinate system, and for illuminant C, are shown in Table LXXXVI. The reflection values for the wavelengths listed are read off from the reflection curve obtained with the spectrophotometer. These values are added up separately for each of the three primaries. Each of the three sums is multiplied by

TABLE LXXXVI

SELECTIVE ORDINATE METHOD OF HARDY

Red Primary		Green Primary		Blue Primary	
Selected Wavelength	*R*	Selected Wavelength	*R*	Selected Wavelength	*R*
435.5	72.6	489.4	86.6	422.2	66.3
461.2	81.0	515.1	89.1	432.0	70.8
544.2	90.2	529.8	89.8	438.6	73.4
564.0	90.8	541.4	90.1	444.4	75.8
577.3	91.1	551.7	90.5	450.2	77.8
588.7	91.4	561.9	90.7	455.9	79.5
599.5	91.6	572.5	91.0	462.0	81.2
610.8	92.0	584.8	91.3	468.8	82.9
624.0	92.5	600.7	91.6	477.8	84.6
646.2	93.3	627.1	92.6	495.3	87.4
Sums	886.5	.....	903.3	.....	779.7
Factors	0.09804	.....	0.10000	.....	0.11812
Products	86.91	.....	90.33	.....	92.10
Trichromatic coefficients	0.3227	..	0.3354	..	0.3419

a factor, 0.09804 for the red primary, 0.10000 for the green primary, and 0.11812 for the blue primary. The value 0.1 has been chosen for the green primary in order that the product may give the brightness of the sample directly. The trichromatic coefficients for each of the three primaries are finally calculated in the usual manner and expressed as fractions of 1. This method of computation is illustrated in Table LXXXVI for the same white sugar as before. The reflection values (*R*) shown, in percentage, have been interpolated from column 2 in Table LXXXV.

The trichromatic coefficients obtained by this method agree with those

found by the weighted ordinate method (Table LXXXV) within one unit in the fourth decimal place, an excellent check. The brightness is found to be 90.3, against 90.2, also in good agreement. The dominant wavelength and the purity are again found from the graph or by calculation, as previously explained.

The color of a sugar solution may be expressed in the monochromatic system in a perfectly analogous manner as the color of a solid sugar, by substituting transmittancy values found by spectrophotometric analysis for the reflection values.

The photoelectric spectrophotometer of Hardy (p. 590) has an integrating attachment which gives the trichromatic analysis without further calculation.

Reflection Value of White Sugars.[143] Although the brightness, purity, and dominant wavelength of a sugar completely specify its color, it is frequently desired to express the " whiteness " of a solid white sugar in a single figure. A long series of comparisons made at the Java Sugar Experiment Station between the monochromatic analysis of white sugars and their whiteness as perceived by trained observers has shown that the dominant wavelength varies within narrow limits only and has no noticeable effect on the appearance of a white sugar. The whiteness can be expressed simply by deducting one-half of the colorimetric purity from the brightness. For a white sugar of 90.2 per cent brightness and a colorimetric purity of 14 the reflection value is thus 90.2 — 7, or 83.2.

Reduced Reflection Value of White Sugars. When the color of a sugar is determined on the basis of the transmittancy of its solution in water the grain size of the sugar has naturally no effect on the result. But the color of a sugar as perceived by the eye varies with the crystal size, a coarse-grained sugar appearing less white than a fine powder of the same sugar. The Java Sugar Experiment Station has therefore decided to express the reflection value of white sugars in terms of a standard grain size, for which 1 mm. has been chosen. This corrected reflection value is called the " reduced " reflection value. It has been found that for each 0.1 mm. decrease in grain size the reflection value increases one unit, and vice versa. Therefore, if the specific grain size (see Chapter XVII) is less than 1 mm., the corresponding correction is subtracted from the reflection value found, and if the grain size is greater than 1 mm., the correction is added. Taking again the above example of a sugar with a reflection value of 83.2, and supposing that the specific grain size of this sugar is 0.64 mm., the reduced reflection value is 83.2 — [10 $\times$ (1.00 — 0.64)], or 79.6.

[143] Private communication from K. Douwes Dekker.

Yellow and brown sugars vary so much in the dominant wavelength that it is not possible to express their color in a single figure.

Keane and Brice (p. 608) have found an empirical relationship between the " apparent color index," I_c, of a white sugar, measured upon its solution, the average grain size of the solid sugar in millimeters, g, and the reflection value R as found with their photoelectric reflection meter, using white light and an angle of reflection of 45°. The equation is as follows:

$$R = 97.02 - 0.206\, I_c - 14.3\, g$$

The calculated R values show only fair agreement with those actually observed.

The spectrophotometric method of the Java Sugar Experiment Station for determining the color of solid sugars is the only one developed so far that is based on strictly scientific principles. The usual reflection meters, using white light (p. 600), give only comparative values and do not permit expression of the results in the monochromatic system.

MEASUREMENT OF TURBIDITY

Suspended matter or turbidity plays an important part in the manufacture of sugar. That present in the sirups of the raw-sugar factory affects the filterability of the product. Turbidity is also an important criterion in judging the quality of refinery liquors and of refined sugars.

Turbidity, like color, is regarded from different viewpoints by the chemist and by the physicist. The chemist is interested primarily in the concentration of the suspended material and its effect on factory operation; the physicist is concerned more with the optical properties of turbid solutions.

Often the quantity of suspended matter can be determined by filtration and weighing, but the amount found by this method depends on the pore size of the filtering medium with respect to the size of the suspended particles. However, when the turbidity is slight and is caused by very small particles, it is necessary to make use of optical methods. These can be only briefly discussed here; for more detailed information the chemist is referred to the literature on the subject.[144]

A molecular dispersion is optically void; it absorbs visible light, but does not reflect or scatter it, except for reflection at the surface. Particles very small compared to the wavelength of light cause scattering, while larger particles reflect and refract light. In both cases a portion

[144] Wells, *Chem. Rev.*, 3, 331 (1927); Teorell, *Kolloid-Z.*, 53, 322 (1930); 54, 58, 150 (1931); Landt and Witte, *Z. Ver. deut. Zucker-Ind.*, 84, 450 (1934); Sauer, *Z. tech. Physik*, 12, 148 (1931).

of the light is absorbed also. Turbidity measurements may therefore be based either on the proportion of the light transmitted, by methods analogous to those described in the preceding section of this chapter, or on that of the scattered and reflected light, given by the intensity of the so-called Tyndall beam. Methods utilizing the Tyndall-beam intensity are especially indicated for low turbidities which cause such slight absorption that it cannot be accurately measured.

The laws governing the optical properties of turbid solutions, even in colorless media, are more complex than those for molecular solutions. A complete theory, assuming perfectly diffused incident light, has been developed by Channon, Renwick, and Storr.[145] Formulas for both the optical density (—log **T**) and for the Tyndall-beam intensity, in relation to depth and concentration, have been given by Wells. This author has shown that the optical density increases more slowly than the depth or concentration because part of the light is lost by scattering or reflection. The rigorous equations are rather involved, but simpler relationships have been found experimentally to hold over restricted ranges of depth and concentration. Over a fairly wide range, and for not too concentrated dispersions, the optical density is a power function of the depth or concentration, as shown by the formula

$$D_x \div D_1 = x^n$$

where D_x is the optical density at thickness or concentration x, D_1 that at unit thickness or concentration, and n an exponent which is numerically less than unity.

The Tyndall-beam intensity is an even more complicated function of the depth or concentration of turbid solutions with colorless media. Wells gives an equation for that relationship also, but it contains eight constants and is too cumbersome for practical use. Within limited ranges the simpler power formula given above, with the Tyndall-beam intensity substituted for the optical density, affords close approximation to the experimental data in many cases. At high concentrations both optical density and Tyndall-beam intensity reach a maximum and then decrease, owing to multiple reflection.

The theoretical assumptions which have been made in establishing the equations mentioned above are usually not realized in the various instruments that have been designed for measuring the transmittancy or Tyndall-beam intensity of turbid solutions, and it is generally necessary to calibrate each instrument and type of solution, and to derive equations or draw curves to fit the experimental data. In practice it is frequently found that the power formula given previously applies over

[145] *Phot. J.*, **58**, 121 (1918).

fairly wide ranges, and in some cases even the Beer-Lambert law may hold within the experimental error. With molecular solutions the width or shape of the cells used is of little consequence, but with turbid solutions there are edge effects which influence the results obtained.

In the preceding discussion it has been assumed that monochromatic light is used, and that the turbidities to be measured differ only in concentration, but not in their nature, either chemical or physical. These characteristics, particularly the size and shape of the particles, have an important effect on the optical properties of the dispersion. Lord Rayleigh has shown that for light-scattering particles the ratio R of the Tyndall-beam intensity to that of the incident beam may be expressed by the following formula, somewhat simplified in form:

$$R = \frac{k \times N \times V^2}{\lambda^4}$$

where k is a constant depending on the refractive indices of the dispersoid and of the dispersion medium, and on the distance and the angle between the scattered and incident beams; N is the number of the particles; V, their total volume; and λ, the wavelength. Since $N \times V$ represents the concentration, it is seen that the Tyndall ratio varies directly with V, or with the cube of the diameter, and inversely with the fourth power of the wavelength. For larger particles, which reflect rather than scatter light, the Tyndall ratio increases at a slower rate than the cube of the diameter of the particles. At the same time the exponent of the wavelength becomes less than 4, decreasing with growing particle size. The optical density varies with particle size in a similar manner as the Tyndall ratio.

The light scattered by extremely small particles is completely polarized at a right angle to the incident beam. With increase in particle size the ratio of polarized light to total light decreases. The degree of polarization is therefore a sensitive criterion of particle size.

No general theory has been developed for the optical properties of colored dispersoids in colored media, such as are usually encountered in the sugar industry, but because of the practical importance of the subject various attempts have been made to solve the problem empirically.

Two types of methods have been used: in one of these the turbidity is removed by filtration; in the other, unfiltered solutions are employed.

Method of Balch. As an example of the first group of methods that of Balch[146] will be described. A 60-Brix solution of the sugar or other

[146] *Ind. Eng. Chem., Anal. Ed.*, 3, 124 (1931).

product is prepared as shown on p. 602 and filtered with the aid of Filter-Cel (p. 603). One absorption cell is filled with unfiltered solution, another of the same dimensions with filtered solution, and the transmittancy of the former with respect to the latter is measured in a spectrophotometer at any desired wavelength, such as 560 mμ. Balch found that Beer's law does not strictly hold for the turbidity, but for practical purposes it is simplest to express it in terms of the specific absorptive index, —log t, in the same way as is done for coloring matter. Even if Beer's law did apply exactly for each particular kind of turbidity, the —log t found for one type would not express the same turbidity concentration as the —log t found for another type of different particle size and shape.

Some investigators have used Hyflo Supercel instead of Filter-Cel, and ultrafilter membranes are also being employed to separate the turbidity from the coloring matter. Zerban and Sattler have shown (p. 605) that Celite Analytical Filter Aid, asbestos, and finely powdered silica gel remove not only turbidity, but also varying amounts of coloring matter, depending on the pore size of the filtering medium and the particle size of both turbidity and coloring matter. Comparable results can therefore be obtained only by strictly standardizing each procedure. But the filtration method has proved useful as an empirical process for routine comparisons, and various modifications of it are used not only in America, but also in European and other countries.

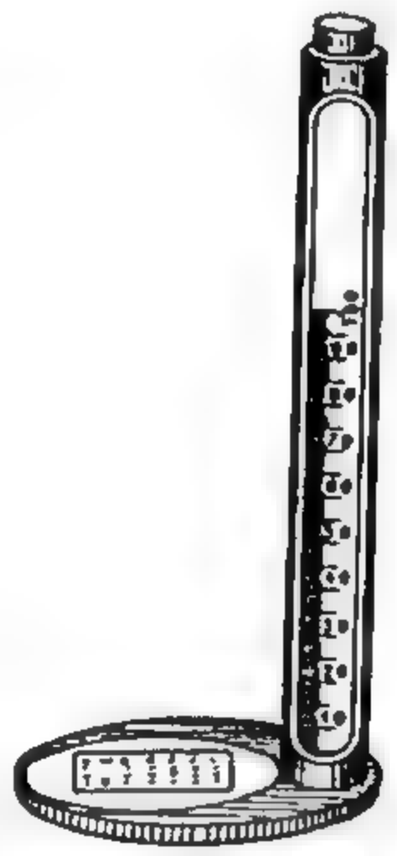
The other group of methods, employing unfiltered solutions, may be further subdivided into four classes. Either transmitted light may be used and (1) the transmittancy determined photometrically, or (2) the degree of turbidity may be judged by the criterion of complete extinction. Instruments based on these two principles are properly called "turbidimeters" (Wells's nomenclature). Or the Tyndall-beam intensity may be measured, either (3) by comparison with a standard turbidity, as in "colorimetry" (nephelometers), or (4) by photometry (tyndallmeters).

Transmittancy Method. Method 1 is applicable, strictly speaking, only to turbidities in colorless media, and is therefore not suitable for most sugar products. However, if only approximate values are needed, the method of Keane and Brice (p. 607), or that of Nees (p. 608) may be used for determining the turbidity in white sugars. According to Keane and Brice the coloring matter in white sugars absorbs so little light at the red end of the spectrum that any absorption found may be ascribed to the turbidity alone. The turbidity is thus measured by $1 - T_r$, and the "turbidity index" is 100 times that quantity. Nees found, however, that the absorption in the red end of the spectrum is

not due to turbidity alone, but partly also to coloring matter, and that the turbidity values found by the method of Keane and Brice are too high. Nees recommends his own method for both color and turbidity determination. But, as has been explained on p. 609, both methods must be used with caution.

Extinction Criterion. The second method is used extensively in water analysis. The depth at which an object, like a wire, lowered into the column of liquid, or a small light placed below a column of variable height, just becomes invisible, serves as a measure of the turbidity. This procedure is to some extent independent of the color of the solution, but is subject to considerable variation in results, due to differences in the acuity of vision of different observers.

Kopke Turbidimeter. An apparatus based on this principle, Fig. 257, was introduced in the sugar industry by Kopke[147] and is extensively used in Hawaii and the Philippines. It consists of a white porcelain disk the surface of which is ruled with black cross lines. At the side of the disk a glass tube, about 15 cm. long and open at both ends, is mounted vertically. The tube is graduated in millimeters up to 10 or 12 cm., the 0 point being level with the top of the porcelain disk. Standard illumination must be employed for the measurements. A 75-watt blue daylight lamp, with a 10-inch conical shade, is placed, in a dark room, so that the lower edge of the shade is 10 inches above the table. The sample, contained in a cylindrical glass jar, is placed as nearly under the edge of the shade as will permit making the observations directly from above. The turbidimeter is slowly lowered into the solution, in a vertical position, with the plate touching the side of the container nearest the light source. When the ruled lines on the porcelain disk just disappear, the top of the glass tube is closed with the finger, the tube is withdrawn, and the scale read. Several observations are made and the results averaged. The scale reading gives the " clarity," which is the reciprocal of the turbidity, in millimeters.



(Courtesy of Eimer and Amend.)

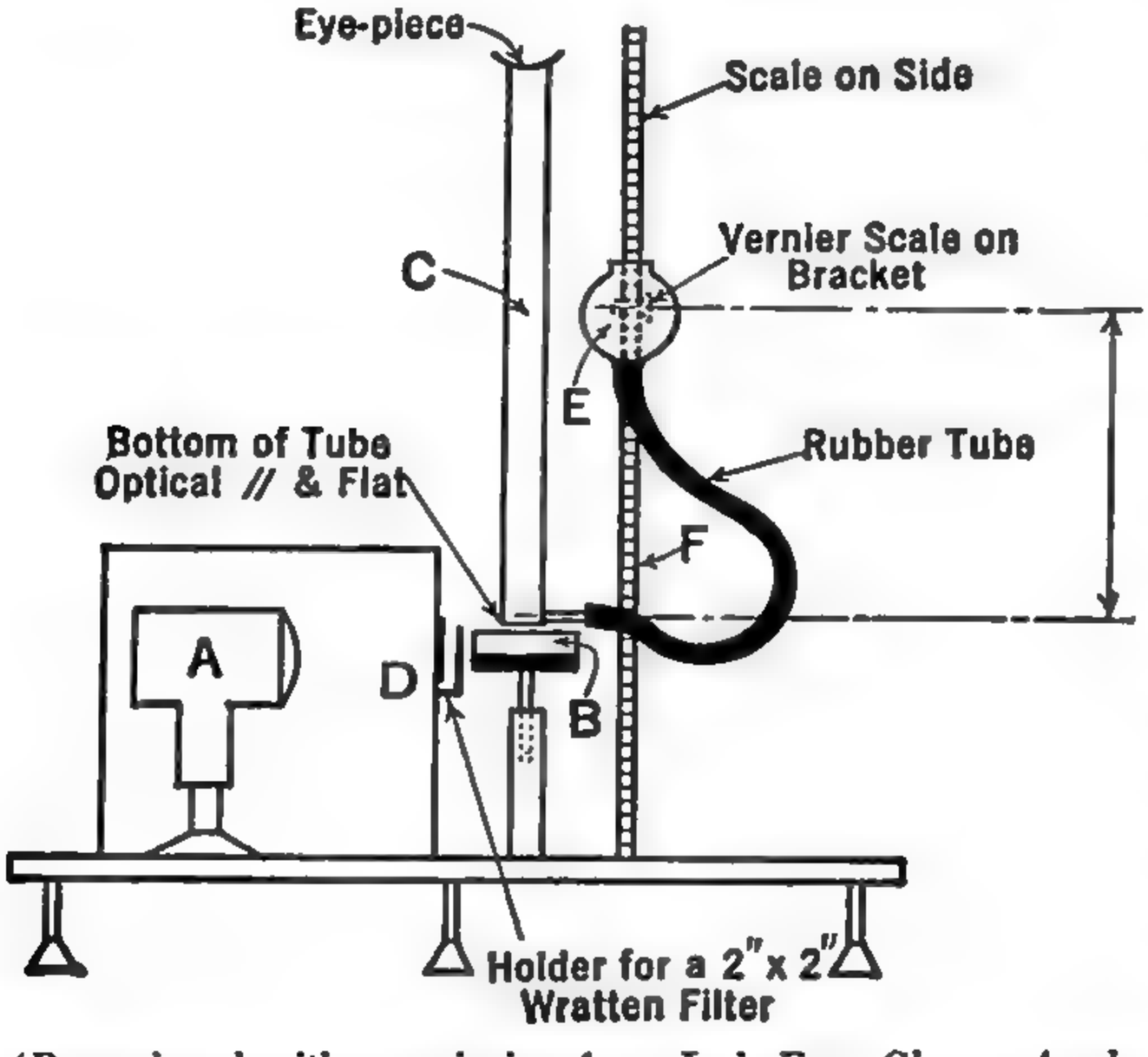
FIG. 257. Kopke turbidimeter.

King,[148] who has made a careful investigation of the Kopke turbidimeter, found that the readings are influenced by the nature of the product examined, by the density of the solution, by particle size, by the color of the solution, and by other factors; also that the subjectivity of the

[147] *Facts About Sugar*, 23, 177 (1928); " Methods of Chemical Control," Assoc. of Hawaiian Sugar Technologists, pp. 19 and 52, 1931.

[148] *Louisiana Planter*, 82, 261 (1929).

measurements precludes uniformity in the results obtained by different observers. He concluded that the instrument is of little value for the determination of turbidity concentration unless all these factors are duly considered, and he suggested standardization of the apparatus with artificial suspensions in media of varying color concentration.



(*Reproduced with permission from Ind. Eng. Chem., Anal. Ed., 2, 248.*)

FIG. 258. Turbidimeter of Ingersoll and Davis.

A device in which the disappearance of a Tyndall beam is used as the extinction criterion, Fig. 258, has been described by Ingersoll and Davis.[149] A horizontal Tyndall beam of 1-mm. thickness is produced at the surface of the liquid in cell B, $4 \times 4 \times 1$ cm. deep, and this overcomes the effect of color to a large extent. A measuring tube C, connected by rubber tubing with bulb E, and filled with concentrated potassium dichromate solution is placed above the cell, and the depth of dichromate solution at which the Tyndall beam just becomes invisible is determined. The primary light beam from lamp A is filtered through a red filter D (Wratten No. 29 F). In the practical range of the instrument the turbidity concentration was found to be directly proportional to the depth of the dichromate solution at the extinction point, except at very low turbidities. The exact effect of the color of the solutions has not been investigated.

Nephelometers. In the nephelometric method the Tyndall-beam intensity of the unknown is matched with that of a standard suspension by varying the height of the latter, as in ordinary colorimetry. The same type of instrument is employed, with the important difference that the colorimeter cups are not illuminated from below but by a horizontal beam of light, striking the cups at a right angle and scattered or reflected by the suspended particles. A curve is drawn, showing the relation between height and concentration of the standard solution when the two halves of the field match exactly, and the concentration of the unknown can be read off from the curve, provided that

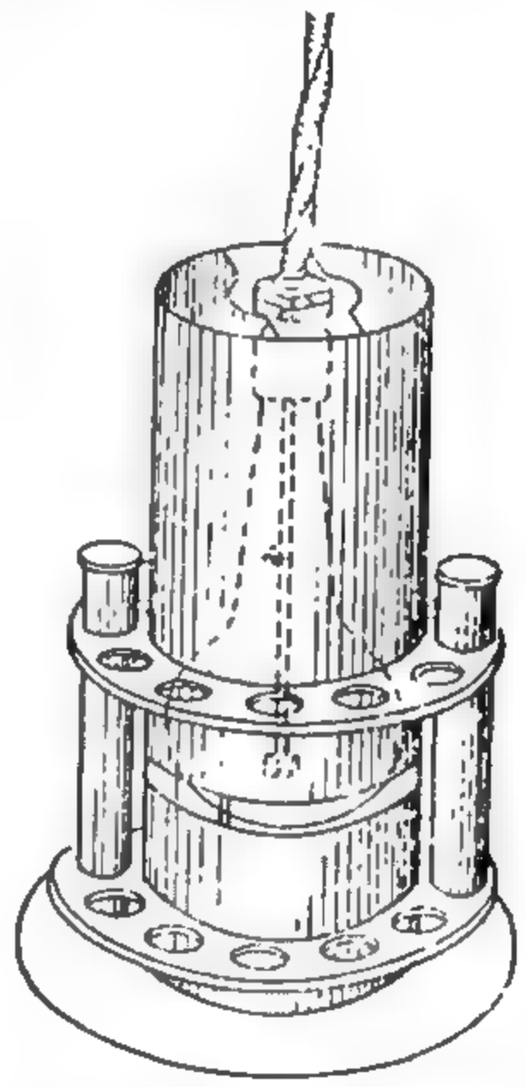
[149] *Ind. Eng. Chem., Anal. Ed.*, **2**, 248 (1930).

the nature of the particles in the unknown is the same as that of the standard.[150]

The nephelometric method is not used much for sugar products, because of the difficulties caused by the presence of coloring matter, varying not only in quantity but also in tint.

Tyndallometry. In this type of methods the intensity of the Tyndall beam is determined photometrically in various ways. For approximate estimations a series of standards may be placed in test tubes illuminated laterally by a strong beam of light, and the unknown in another test tube matched with the standards, by observation at an angle to the incident beam, in a dark room. For more accurate measurements instruments are used by which the intensity of the Tyndall beam of the sample is compared photometrically with that of the incident beam.

Turbidiscope of Horne and Rice. A simple apparatus for estimating the turbidity of liquors in the sugar refinery by observation of the Tyndall beam has been described by Horne and Rice.[151] Its construction may be seen from Fig. 259. It consists of a sheet-metal cylinder inside of which a powerful incandescent lamp is suspended, the socket being fastened to the top of the cylinder in such a way that air can pass freely through the apparatus. Another cylinder, of the same diameter as the first, is attached to it by means of three strong wires, leaving an annular free space, about 1 cm. high, for the light to pass through. A ring, with a number of holes to accommodate test tubes, is fastened around each of the two cylinders, and the test tubes rest on a third, solid ring at the bottom. The metal surfaces are painted black to prevent light reflection and to minimize stray light. The liquors to be examined are placed in the test tubes, and the Tyndall beam is observed in a dark room. The turbidities of liquors of the same color can be readily compared, and for approximate estimations a standard turbidity scale of known concentrations may be prepared, with coloring matter added in the case of colored liquors. Lindfors[152] recommends for this purpose a suspension of 0.5 g. bentonite, rubbed in a mortar with small quantities of water and diluted



(Reproduced with permission from Ind. Eng. Chem., 16, 626.)

FIG. 259. Turbidiscope of Horne and Rice.

[150] For a detailed description of nephelometers and their use see Yoe and Kleinmann, "Photometric Chemical Analysis," Vol. II, "Nephelometry," 1929.

[151] *Ind. Eng. Chem.*, 16, 626 (1924).

[152] *Ind. Eng. Chem.*, 17, 1155 (1925).

to 500 ml. with the addition of 0.2 to 0.4 g. of gum arabic to act as a protective colloid. The suspension is allowed to stand for 24 hours, and 250 ml. is decanted from the sediment. This stock suspension is further diluted as required. Caramel solution is added to the standards, to match the color of the sample to be measured.

Tyndallmeters. The first tyndallmeter was designed by Mecklenburg and Valentiner.[153] In this instrument the primary light is split into two beams. One of these, with a cross section of 1 cm.^{2}, passes horizontally through the turbid solution contained in a large cubical glass cell, open on top. The light passing upward from the Tyndall beam in the solution is directed toward a Lummer-Brodhun cube. The

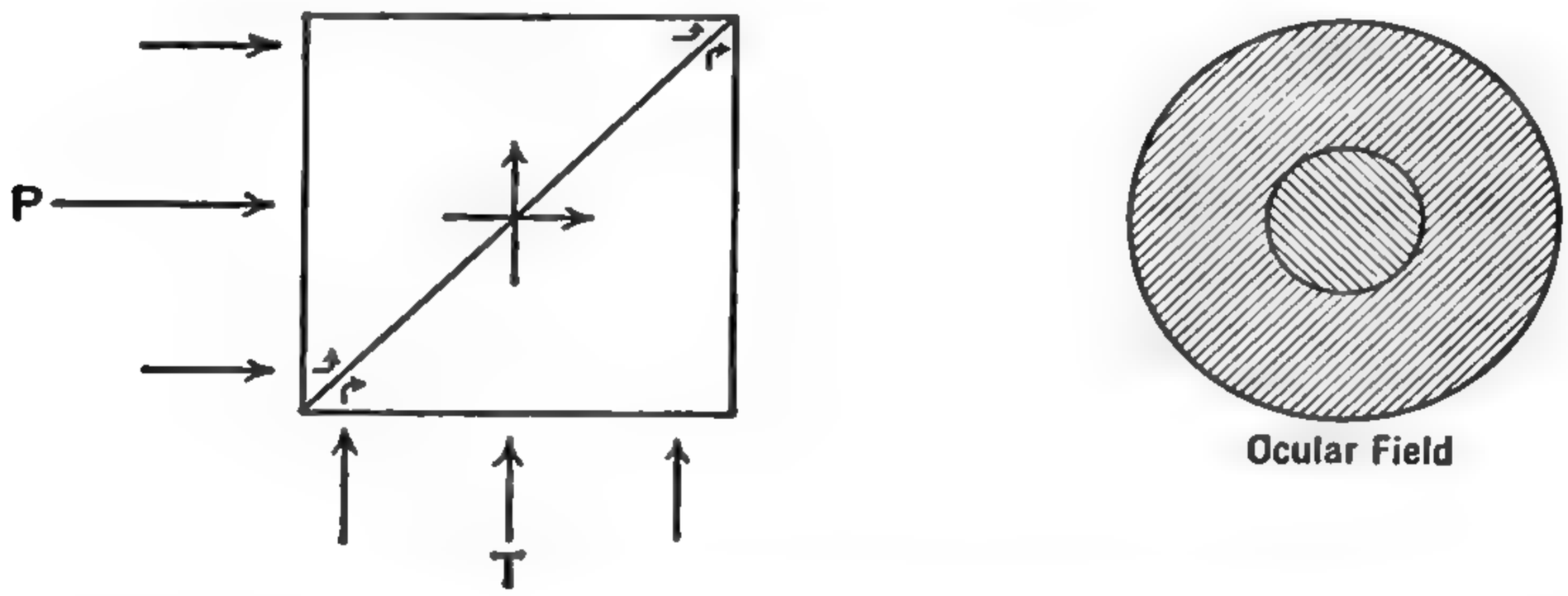


FIG. 260. Showing path of light beams through Lummer-Brodhun cube.

other half of the primary beam is reflected, by a system of mirrors and prisms through a polarization photometer, and enters the Lummer-Brodhun cube horizontally, at a right angle to the Tyndall beam. The effect of the Lummer-Brodhun cube may be understood from Fig. 260. This device consists of two right-angle prisms which are placed together with their hypotenuses touching each other, but they are cemented together with Canada balsam only in their central portion. Hence both the primary beam and the Tyndall beam pass unobstructed through the central portion, but are totally reflected at the outer portion, as shown by the arrows. As a consequence the Tyndall beam forms the outer circle of the ocular field, while the primary beam appears in the inner circle. The photometer drum is turned until the two circles match in brightness, and the reading is taken and converted into the Tyndall ratio. Approximately monochromatic light is obtained by the insertion of color filters. The cell containing the sample can be moved vertically and laterally by means of micrometer screws, so that

[153] *Z. Instrumentenk.*, **34**, 209 (1914).

the effect of varying the position of the Tyndall beam within the liquid may be studied.

A similar tyndallmeter has been designed by Cummins, Badollet, and Miller.[154] It has a neutral wedge instead of a polarization photometer. A red filter is used to furnish nearly monochromatic light. A small cubical cell, open at the top, is employed. The Tyndall beam is a very thin pencil of light, and is observed horizontally at a right angle, through the front wall of the cell, and not from above, as in the instrument of Mecklenburg and Valentiner. The position of the Tyndall beam within the cell is varied and at the same time measured by a micrometer movement of the cell platform. The intensity of the beam is expressed in foot-candles and is considered to be directly proportional to the turbidity concentration, regardless of the size and other characteristics of the particles, but a Nicol prism may be placed between the Tyndall beam and the eyepiece to determine the degree of polarization and hence the average particle size. The color of the solution is corrected for by measuring the intensity of the Tyndall beam at various distances of the beam from the front wall of the cell holding the solution, and extrapolating to zero thickness of layer where the absorption would also be zero.

The tyndallmeter of Tolman and Vliet[155] employs the Macbeth illuminometer as photometer. This instrument is based on the law stating that the intensity of two light sources varies inversely as the square of their distance from the illuminated object. The Tyndall beam produced in the turbid solution by one light source is matched, by means of a Lummer-Brodhun cube, with the variable intensity of the beam from the illuminometer light source. It is evident that the intensity of the two original light sources must be kept strictly constant, and this is a disadvantage of the method for routine measurements. Cummins and Badollet originally used an apparatus of this type for turbidity measurements in sugar products[156] but later abandoned it in favor of the split-beam principle.

In the tyndallmeter of Hellige[157] the intensity of the Tyndall beam produced in a solution is compared with that of a second beam, from the same light source, passing through a slit the width of which is varied and at the same time measured by means of a graduated drum.

A photoelectric instrument for measuring the Tyndall-beam intensity

[154] U. S. Patent No. 2,045,124, 1936.
[155] *J. Am. Chem. Soc.*, 41, 297 (1919).
[156] *Ind. Eng. Chem., Anal. Ed.*, 5, 328 (1933).
[157] *Intern. Sugar J.*, 38, 277 (1936).

of turbid solutions, in the laboratory or in the sugar factory, has been described by Gillett and Holven.[158]

Pulfrich Tyndallmeter. This instrument, sometimes referred to as a nephelometer, employs the photometric principle but differs from other tyndallmeters in that the intensity of the Tyndall beam is compared indirectly with that of the light source. The apparatus is shown diagrammatically in Fig. 261. The photometer is the same as that used for transmittancy determinations and described on p. 597. The other parts of the instrument are assembled in a housing, mounted on a separate stand so that it may be placed in direct contact with the objective end of the photometer, in order to avoid stray light. The light source is in the rear of the housing, somewhat to one side. The primary beam passes through a square diaphragm and a lens into a cylindrical water

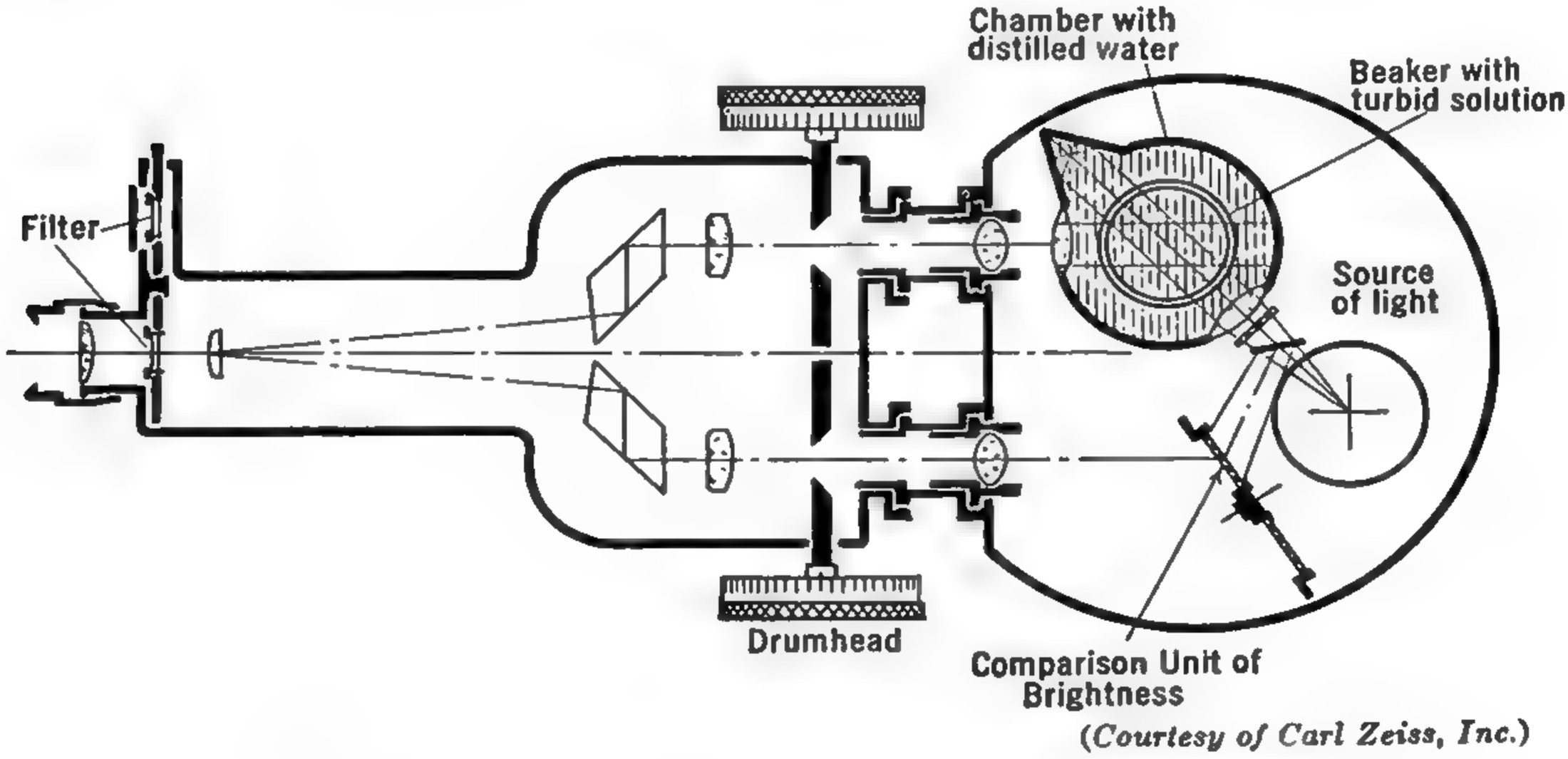


FIG. 261. Showing construction of Pulfrich tyndallmeter.

chamber in the center of which the turbid solution is placed, contained either in a small beaker or in a cell with plane parallel walls. The light scattered or reflected by the particles in the solution passes into the left photometer opening at an angle of 45° to the incident beam. According to Rayleigh's law this angle provides a higher Tyndall-beam intensity than the usual right angle. The comparison beam is furnished by reflection of the primary beam on a glass plate set at an angle. It passes through one of four interchangeable frosted glass disks of different optical densities. From these plates it goes to the right-hand photometer opening. The measurements are made by substitution, the frosted-glass disks being used as auxiliary standards. The beaker or

[158] *Ind. Eng. Chem.*, **28**, 391 (1936).

cell containing the sample is placed in a holder in the water chamber, and the brightness is matched against the glass disk the intensity of which is nearest to that of the sample. Then the sample is removed, a standard turbid glass block is substituted for it, and this is now measured against the same glass disk as was used for measuring the sample. The ratio between the intensity found for the sample and that for the standard block gives a relative measure of the Tyndall-beam intensity of the sample. The Tyndall-beam intensity of this glass block, for 1-cm. thickness, with respect to the intensity of the incident light, has been determined by a separate measurement, and the value thus found is furnished by the manufacturers of the instrument. This makes it possible to reduce the results obtained with different standard blocks to an absolute basis. Readings may be taken in different regions of the spectrum by interposing a blue, green, or red color filter. A correction must always be applied for the turbidity of the water used for dissolving the sugar product.

Method of Landt and Witte for Determining the Turbidity of Sugar Solutions. Landt and Witte[159] have used the Pulfrich instrument for measuring the absolute turbidity of sugar solutions, on the basis of theoretical considerations formulated by Sauer.[160] The formula given by them is as follows:

$$\text{Absolute turbidity } S = A \times f_k \times D \times t$$

where A is the relative Tyndall-beam intensity of the solution, that of the standard block being taken as unity; f_k is a factor to correct for light absorption by the turbid solution; D is a factor to correct for the thickness of the effective Tyndall-beam layer; and t is the absolute turbidity of the standard block. The unit of absolute turbidity is defined by Sauer as the turbidity causing the same amount of light to be radiated in the direction of the observation by a layer 1 cm. thick, as would be radiated in this same direction if the total primary light were scattered uniformly in all directions.

The following example, taken from the work of Landt and Witte, illustrates the method of calculation. A 45-Brix solution of a beet raw sugar was prepared and filtered through filter paper to remove accidental coarse particles. The solution was filled into a cell of 5.1-mm. thickness and measured with the green filter interposed.

With the right drum set at 100, the left drum gave a reading of 29.3 against glass disk No. 1. The Tyndall-beam intensity of the sample is therefore 100 × 100/29.3, or 341.3 per cent of the intensity of the

[159] *Z. Ver. deut. Zucker-Ind.*, **84**, 450 (1934).
[160] *Z. tech. Physik*, **12**, 148 (1931).

comparison beam. The water alone, measured against glass disk No. 1, gave a reading of 7.0 on the right drum, the left drum being set at 100. The relative Tyndall-beam intensity of the water is thus 7.0 per cent, which, deducted from that of the sample, gives a corrected relative Tyndall-beam intensity of 334.3 per cent for the sample. The intensity of the standard glass block was similarly found to be 1136 per cent, relative to glass disk No. 1. Hence the turbidity of the sample relative to that of the glass block is 334.3 ÷ 1136, or 0.294. In a similar experiment with glass disk No. 4, a value of 0.286 was found. The average A is therefore 0.290.

The transmittancy of the same solution in a 1-cm. cell, under the green filter, was 0.525 (52.5 per cent), which gives k (—log T for 1 cm.) equal to 0.280. The corresponding f_k is found from Table LXXXVII for cells of 1-, 2.5-, 5-, and 10-mm. thickness, or from Table LXXXVIII for beakers of 26- and 36-mm. diameter, respectively.

The formula for calculating f_k, for varying k and varying cell thicknesses d, has been derived by Sauer from theoretical considerations, and is as follows:

$$f_k = \frac{kd\,(\sqrt{2} - 1) \times 2.30259}{10^{-kd}\,\{1 - 10^{-kd\,(\sqrt{2}-1)}\}}$$

When the logarithms of f_k are plotted against the k values at constant thickness, a nearly straight line is obtained, starting at $f_k = 0$ and $k = 0$, and satisfying approximately the equation

$$f_k = b^k$$

where b is a constant showing slight fluctuations.

In the above example the f_k for a 5.1-mm. cell and a k value of 0.280 is found, by interpolation of the figures in Table LXXXVII, to be 1.475.

D equals the ratio of the effective thickness of the standard block, 16.3 mm., to that of the vessel used for the measurements. The effective thickness of the 26- and 36-mm. beakers is also 16.3 mm., and D therefore equals 1. The effective layer of the plane parallel cells equals their thickness, and D for the 5.1-mm. cell is thus 16.3 ÷ 5.1 = 3.196.

The value of t for the standard block used by Landt and Witte was 0.0193 for the green filter.

Substituting the values found for A, f_k, D, and t in the formula of Landt and Witte, given above, we obtain for the 45-Brix solution of the sugar examined

$$\text{Absolute turbidity (green filter)} = 0.290 \times 1.475 \times 3.196 \times 0.0193$$
$$= 0.026$$

TABLE LXXXVII

VALUES OF f_k FOR DIFFERENT VALUES OF k AND d

Plane Parallel Cells

k 1-mm. cell	k 2.5-mm. cell	k 5-mm. cell	k 10-mm. cell	f_k
0.10	0.04	0.02	0.01	1.030
0.20	0.08	0.04	0.02	1.061
0.30	0.12	0.06	0.03	1.087
0.40	0.16	0.08	0.04	1.120
0.50	0.20	0.10	0.05	1.149
0.60	0.24	0.12	0.06	1.181
0.70	0.28	0.14	0.07	1.216
0.80	0.32	0.16	0.08	1.250
0.90	0.36	0.18	0.09	1.284
1.00	0.40	0.20	0.10	1.319
1.50	0.60	0.30	0.15	1.514
2.00	0.80	0.40	0.20	1.731
3.00	1.20	0.60	0.30	2.286
4.00	1.60	0.80	0.40	3.02
5.00	2.00	1.00	0.50	3.97
6.00	2.40	1.20	0.60	5.22
7.00	2.80	1.40	0.70	6.87
8.00	3.20	1.60	0.80	9.02
9.00	3.60	1.80	0.90	11.83
10.00	4.00	2.00	1.00	15 19

TABLE LXXXVIII

VALUES OF f_k FOR DIFFERENT VALUES OF k AND d

Beakers

k	f_k 26-mm.	f_k 36-mm.	k	f_k 26-mm.	f_k 36-mm.
0.0004	1.002	1.003	0.0217	1.133	1.172
0.0022	1.013	1.016	0.0261	1.162	1.210
0.0043	1.026	1.033	0.0304	1.191	1.250
0.0087	1.051	1.066	0.0347	1.222	1.290
0.0130	1.078	1.100	0 0391	1.252	1.332
0.0174	1.105	1.136	0 0434	1.284	1.375

Method of Zerban, Sattler, and Lorge for Determining Both Turbidity and Color. These authors[161] found that the method of Landt and Witte is satisfactory when applied to white sugars. But if any appreciable quantities of coloring matter are present, the f_k values of Sauer, when based on the transmittancy of the turbid solution, give too high figures. For example, sugar solutions containing the same

[161] *Ind. Eng. Chem., Anal. Ed.*, 3, 326 (1931); 6, 178 (1934); 7, 157 (1935); 8, 168 (1936); 9, 229 (1937); 10, 9 (1938).

amount of coloring matter, and turbidities in the known proportions of 5 : 4 : 3 : 2 : 1, gave by the method of Landt and Witte turbidities in the proportions of 15.86 : 9.07 : 5.53 : 2.90 : 1.

By measuring the transmittancy as well as the Tyndall-beam intensity of sugar sirups of 60 Brix and containing known relative amounts of coloring matter and turbidity, in cells of 2.455-mm. thickness, the following empirical relationships were found to hold:

$$R = \frac{a \times N}{b^c} \tag{1}$$

$$-\log \mathrm{T} = mN + nC \tag{2}$$

where R is the Tyndall-beam intensity in percentage of the standard block ($R = 100\ A$ of Landt and Witte). The standard block used had an absolute turbidity t of 0.00282 for the green filter; t for the blue and red filters was not known. N is the relative turbidity concentration, C the relative concentration of coloring matter, and —log T is the negative logarithm of the observed transmittancy at the dry-substance concentration and cell thickness specified above; a, b, m, and n are constants the values of which were calculated from the experimental data. Beer's law was found to hold approximately, for both N and C, over a five-fold range of concentration, and both may therefore be expressed as negative logarithms of the transmittancy, for 60 Brix and 2.455-mm. thickness:

$$-\log \mathrm{T} = N + C \tag{3}$$

Under the experimental conditions used the values of the logarithms of the constants a and b are as follows:

	Blue Filter	Green Filter	Red Filter
log a	3.1306	3.7755	4.4056
log b	1.4255	1.2328	1.1657

With other standard blocks the R values for the green filter must first be corrected by multiplying by the absolute turbidity of the block and dividing by 0.00282. Cells of approximately 2.5-mm. thickness must be used, and the observed R and —log T values must be reduced to 2.455 mm.

It is noted that equation 1 is of the same general form as Sauer's equation for the absolute turbidity (p. 631). Since $A = 0.01\ R$,

$$S = 0.01\ R \times f_k \times D \times t \tag{4}$$

$$N = R \times b^c \times 1/a \text{ (from equation 1)} \tag{5}$$

In the work on white sugars, where the method of Landt and Witte gives correct results for the turbidity, the ratio between the absolute

turbidity S and the turbidity expressed as N was found to be 1.117. Substitution of 1.117 N for S in equation 4, and solving for f_k, disclosed the fact that this correction factor which is approximately equal to b^k (p. 632) is, within the limits of experimental error, also equal to b^C in equation 5, established by experiment. It was thus proved that the correction factor must be based, not on k, the negative logarithm of the transmittancy of the turbid solution, but on C, the negative logarithm of the transmittancy for the coloring matter alone.

The only remaining difference between equations 4 and 5 is in the constants. This is due to the fact that in the former the turbidity is expressed in relation to the incident primary light, while in the latter it is expressed as —log **T**. The ratio between 0.01 $D \times t$ (i.e.,0.01 $\times$ 6.64 $\times$ 0.00282) and $1/a$ (i.e., $1 \div 5964$) is, of course, the same as between S and N, viz., 1.117.

Since f_k is only approximately equal to b^k, it was concluded that b^C, found experimentally, is expressed more exactly by Sauer's theoretical formula for f_k (p. 632), but based on C. This correction factor is termed f_c. Substituting f_c for b^C in formula 5, we obtain

$$N = \frac{Rf_c}{a}$$

Combination with equation 3, $C = -\log \mathbf{T} - N$, gives

$$C = -\log \mathbf{T} - \frac{Rf_c}{a} \tag{6}$$

This value for C is now substituted for kd in Sauer's equation for f_k (p. 632). Sauer's k being expressed as —log **T** for 1 cm., and C as —log **T** for 0.2455 cm., C must first be divided by 0.2455 to reduce it to 1-cm. thickness, and the quotient must then be multiplied by $d = 0.2455$ cm. The net result is C in place of kd. The formula for f_c thus becomes

$$f_c = \frac{\left\{ -\log \mathbf{T} - \left(\frac{Rf_c}{a}\right)\right\}(\sqrt{2} - 1) \times 2.30259}{10^{-\left[-\log \mathbf{T} - \left(\frac{Rf_c}{a}\right)\right]\left\{1 - 10^{-\left[-\log \mathbf{T} - \left(\frac{Rf_c}{a}\right)\right](\sqrt{2}-1)}\right\}}} \tag{7}$$

The physical significance of constant a may be derived from equation 6. When C equals 0 and consequently f_c is equal to unity, a equals $R/(-\log \mathbf{T})$. This relationship makes it possible to check a experimentally, and also to tell whether coloring matter is present in significant amounts, because in its absence the ratio between R and —log **T** should not vary.

In order to calculate C and N from the observed R and —log **T**, equation 7 is solved for varying values of C, which is the —log **T** — (Rf_c/a) term in equation 6, and a tabulation of corresponding f_c values is thus obtained. Substitution of these C and f_c values at specified increments of R in equation 6 yields a table from which C may be found

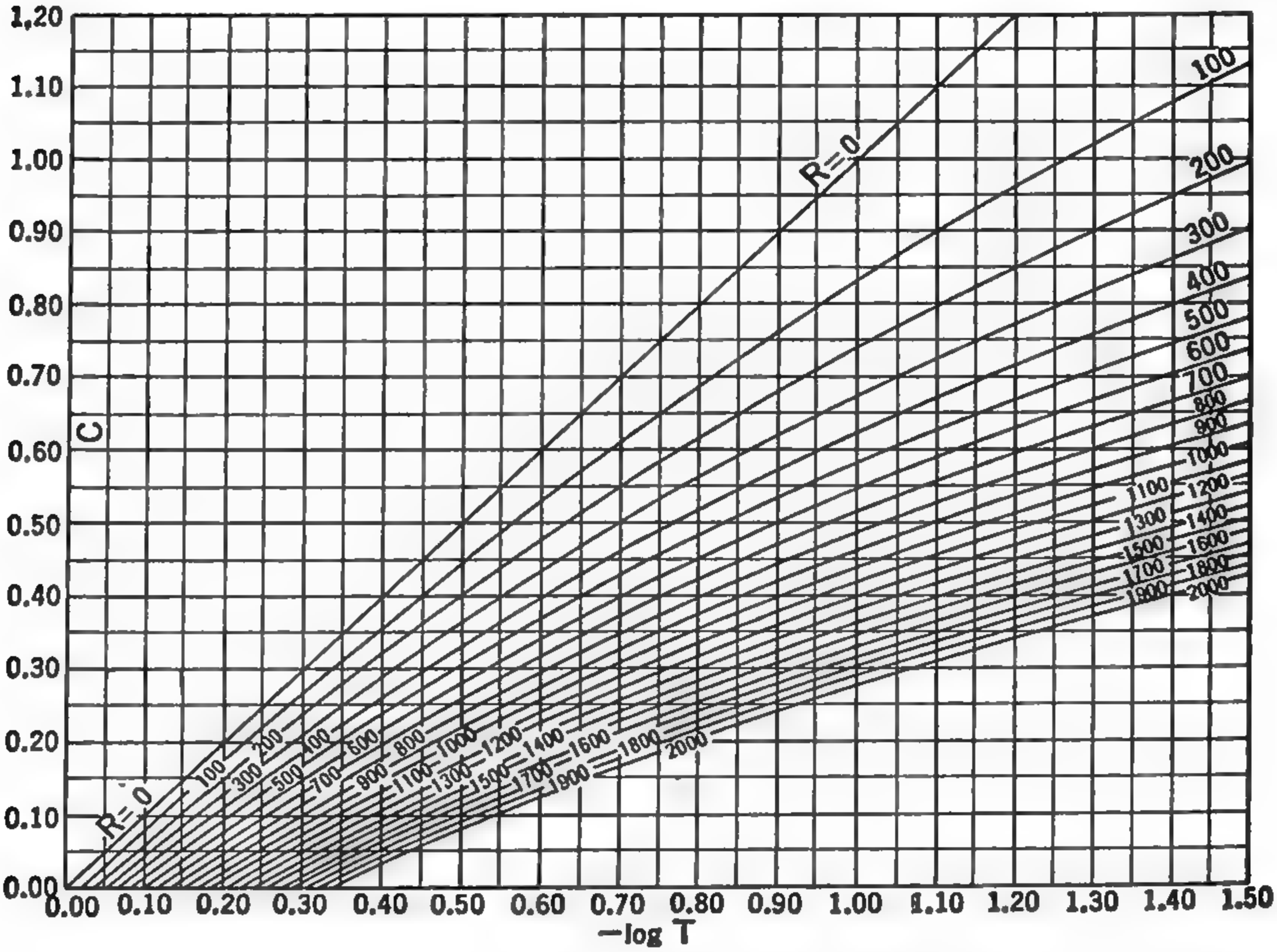


FIG. 262. Curves for finding color concentration from transmittancy and Tyndall-beam intensity; full range for raw and white sugars.

for any pair of observed —log **T** and R values. It is easier to find C from a graph based on the table. Finally, N is obtained by subtracting C from the observed —log **T** of the turbid solution.

A graph covering the entire range of C, —log **T**, and R values for cane sugars is shown in Fig. 262, and an enlarged graph giving only the lower portions of the curves, to be used for white sugars, in Fig. 263. If the graphs are drawn on such a scale that increments of 0.1 C and 0.1 (—log **T**) are equal to 50 mm. in the first graph, and increments of 0.001 C and 0.001 (—log **T**) equal to 50 mm. in the second, the result for C can be read off with sufficient accuracy for any given sugar, raw or white.

If more exact results are desired, interpolation of R is carried out by means of Table LXXXIX, on the basis of the approximate value of C

read from the graph. The use of the table is best explained by an example. A raw-sugar sample examined in a 60-Brix solution, in a 2.455-mm. cell, and with a standard block of absolute turbidity 0.00282, gave $-\log T = 0.57807$, and $R = 917.1$, for the green filter. A glance at the graph shows that C lies between 0.25 and 0.30. The value of

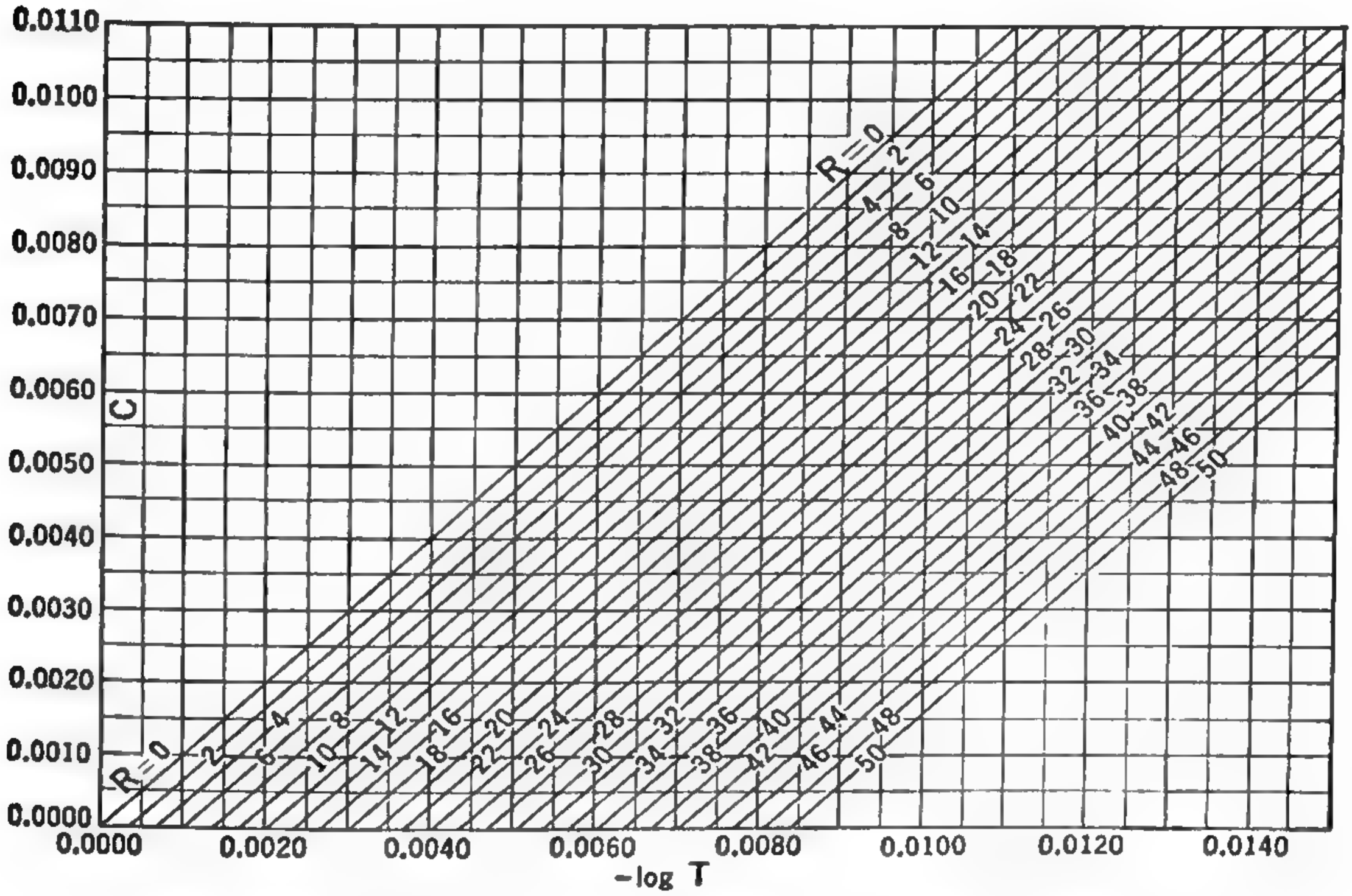


FIG. 263. Enlarged graph of lower portion of Fig. 262, for white sugars.

Rf_c/a for 1 R, at $C = 0.25$, is 0.0003351; hence that for 917.1 R is 0.0003551×917.1, or 0.30732, which added to 0.25 C gives $-\log T = 0.55732$. Similarly, the value of Rf_c/a for 1 R, at $C = 0.30$, is 0.0003847, and that for $R = 917.1$ is 0.35280, which added to 0.30 gives $-\log T = 0.65280$. Then the required value of C is found from the following equation:

$$(C - 0.25) : (0.30 - 0.25) = (0.57807 - 0.55732) : (0.65280 - 0.55732)$$

The result for C is 0.2609. N equals 0.57807 — 0.2609, or 0.3172.

The above interpolation assumes linear relationship between Rf_c/a and C for the small traject between 0.25 C and 0.30 C. The curve shows that this is not quite correct. The aberration, however, is so slight that the result obtained is well within the limits of error of the

photometric data. If the interpolation is made on the basis of the more exact linear relationship between log Rf_c/a and C, the result for C is 0.2611.

TABLE LXXXIX

INTERPOLATION TABLE FOR FINDING C AND f_C FROM — LOG T AND R

C	f_C	$\frac{Rf_C}{a}$ for $R = 1$	C	f_C	$\frac{Rf_C}{a}$ for $R = 1$
0.0000	1.0000	0.0001677	0.1000	1.3200	0.0002213
0.0010	1.0027	0.0001681	0.1500	1.5160	0.0002542
0.0020	1.0055	0.0001686	0.2000	1.7406	0.0002919
0.0030	1.0083	0.0001691	0.2500	1.9986	0.0003351
0.0040	1.0110	0.0001695	0.3000	2.2941	0.0003847
0.0050	1.0138	0.0001700	0.3500	2.6331	0.0004415
0.0060	1.0166	0.0001705	0.4000	3.0214	0.0005066
0.0070	1.0194	0.0001709	0.4500	3.4664	0.0005813
0.0080	1.0222	0.0001714	0.5000	3.9757	0.0006667
0.0090	1.0250	0.0001719	0.5500	4.5598	0.0007646
0.0100	1.0278	0.0001723	0.6000	5.2281	0.0008767
0.0110	1.0306	0.0001728	0.6500	5.9937	0.0010050
0.0120	1.0335	0.0001733	0.7000	6.8699	0.0011520
0.0130	1.0363	0.0001738	0.7500	7.8727	0.0013201
0.0140	1.0391	0.0001742	0.8000	9.0199	0.0015125
0.0150	1.0420	0.0001747	0.8500	10.3329	0.0017327
0.0160	1.0448	0.0001752	0.9000	11.8342	0.0019844
0.0170	1.0477	0.0001757	0.9500	13.5520	0.0022724
0.0180	1.0506	0.0001762	1.0000	15.5154	0.0026017
0.0190	1.0535	0.0001767	1.0500	17.7609	0.0029782
0.0200	1.0564	0.0001771	1.1000	20.3270	0.0034085
0.0220	1.0622	0.0001781	1.1500	23.2604	0.0039004
0.0240	1.0680	0.0001791	1.2000	26.6117	0.0044623
0.0260	1.0739	0.0001801	1.2500	30.4415	0.0051045
0.0280	1.0798	0.0001811	1.3000	34.8136	0.0058376
0.0300	1.0862	0.0001821	1.3500	39.8110	0.0066756
0.0350	1.1023	0.0001848	1.4000	45.5150	0.0076321
0.0400	1.1175	0.0001874	1.4500	52.0280	0.0087242
0.0450	1.1332	0.0001900	1.5000	59.4610	0.0099706
0.0500	1.1488	0.0001926	1.5500	67.9469	0.0113936
0.0550	1.1647	0.0001953	1.6000	77.6286	0.0130170
0.0600	1.1814	0.0001981	1.6500	88.6764	0.0148696
0.0650	1.1978	0.0002008	1.7000	101.1057	0.0169537
0.0700	1.2146	0.0002037	1.7500	115.6513	0.0193928
0.0750	1.2316	0.0002065	1.8000	132.0390	0.0221408
0.0800	1.2491	0.0002095	1.8500	150.7322	0.0252753
0.0850	1.2661	0.0002123	1.9000	172.0380	0.0288479
0.0900	1.2838	0.0002153	1.9500	196.3277	0.0329209
0.0950	1.3017	0.0002183	2.0000	224.0030	0.0375616

C and N having been determined, in terms of —log T, for a thickness of 0.2455 cm., and a concentration of 60 Brix, the results are finally

converted into —log t (specific absorptive index) values in accordance with Lambert-Beer's law as explained on p. 591.

The turbidity may also be expressed in terms of absolute turbidity by means of Sauer's formula (p. 631). But in this case the observed *R*, and not that corrected for the absolute turbidity of the standard block, must be used, because the formula automatically corrects for this. In the example cited above, the f_c corresponding to $C\ (= kd) = 0.2609$ is found from Table LXXXIX to be 2.0630. By substituting this figure in Sauer's formula for *S*, with $A = 9.171$, $D = 6.64$, and $t = 0.00282$, we find the absolute turbidity to equal 0.3542. The ratio between this figure and the *N* found previously is again 1.117.

Neither the transmittancy nor the Tyndall-beam intensity of high-grade white sugars can be measured with sufficient accuracy in cells of only 2.455-mm. thickness. The former should be determined in cells of at least 10-cm. length, and the latter in 26-mm. beakers having an effective thickness of 16.3 mm. The results must then be reduced to a thickness of 2.455 mm. Lambert's law does not hold over such a wide range, and the calculation must be made by the power formula given on p. 622. The value of the exponent was found experimentally to be 0.955. If, for example, the transmittancy is measured in a 100-mm. cell, the —log T found must be multiplied by $2.455^{0.955} = 2.358$, and the product divided by $100^{0.955} = 81.28$. Similarly, if the *R* is measured with the 26-mm. beaker, the *R* found must be multiplied by $2.455^{0.955} = 2.358$, and the product divided by $16.3^{0.955} = 14.376$. If necessary, the *R* thus obtained is corrected for the absolute turbidity of the standard block used. *C* and *N* are then found from the corrected —log T and *R* figures as shown in the following example.

The 60-Brix solution of a white sugar gave, in a 100-mm. cell, with the green filter, a $-\log T$ of 0.1638; hence $-\log T$ for 2.455 mm. $= 0.1638 \times 2.358 \div 81.28 = 0.0048$. The *R* for 16.3-mm. thickness, and a standard block of absolute turbidity 0.00282, was 115.1; *R* for 2.455-mm. thickness $= 115.1 \times 2.358 \div 14.376 = 18.88$. The enlarged graph, Fig. 263, shows that for these values of $-\log T$ and *R* the *C* value lies between 0.001 and 0.002. The value of Rf_c/a (Table LXXXIX), for $C = 0.001$ and 1 *R*, is 0.0001681; hence for 18.88 *R* it equals 0.0032. Similarly, the value of Rf_c/a for 0.002 *C* and 18.88 *R* is found to be also 0.0032. The figure 0.0032 added to $C = 0.001$ gives 0.0042, and added to 0.002 it yields 0.0052. Then

$$(C - 0.001) : (0.002 - 0.001) = (0.0048 - 0.0042) : (0.0052 - 0.0042)$$

Solving for *C* gives 0.0016. *N* equals 0.0048 — 0.0016, or 0.0032.

The absolute turbidity of this sugar can also be calculated directly by Sauer's equation, because in this case the coloring matter is negli-

gible. Sauer's factor f_k, for a cell of 2.455 mm. and a k (for 1 cm.) of $0.0048 \div 0.2455 = 0.0196$, is found from Table LXXXVII to equal 1.014. Then

$$S = 18.88 \times 1.014 \times 6.64 \times 0.00282 = 0.0036$$

This figure, divided by 1.117, gives $N = 0.0032$, checking with the result obtained previously.

The method of Zerban, Sattler, and Lorge offers the great practical advantages that both turbidity and color are found by the use of only one solution, that no filtration is necessary, and that, unlike the methods of Keane and Brice or of Nees, it is applicable to both white and colored products. The turbidity values obtained by this method are usually lower, and the color values higher, than by the filtration methods, but there are exceptions to this rule. The discrepancies in either direction are readily explained by the selective removal of both turbidity and coloring matter through the action of the Filter-Cel or other filtering agent employed, as shown on p. 605.

None of the methods described takes the size, shape, and other properties of the particles into consideration from the quantitative standpoint. There is no uniformity in the turbidity units employed in the different procedures. Cummins and Badollet express the turbidity in-directly, in foot-candles. In view of the fact that light is absorbed selectively by both coloring matter and turbidity, and that these two differ principally in particle size, it appears more logical to use the specific absorptive index as a unit measure for both, as advocated by Balch, and by Zerban, Sattler, and Lorge. The absolute turbidity values of Landt and Witte are readily converted into $-\log T$ and $-\log t$ values as shown on p. 635.

Much research work remains to be done in this field. It is very doubtful whether a simple method can be devised which would give the actual turbidity concentration in terms of mass, regardless of size, shape, color, etc., of the particles in the presence of varying concentrations of coloring matter. It will probably be necessary to combine determinations of transmittancy and Tyndall-beam intensity throughout the spectrum with other physical measurements, such as ultramicroscopic examination, degree of polarization caused by the particles, sedimentation with the ultracentrifuge, and similar observations.

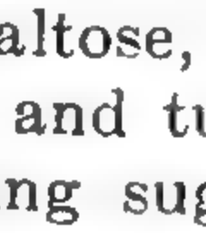
CHAPTER XIII

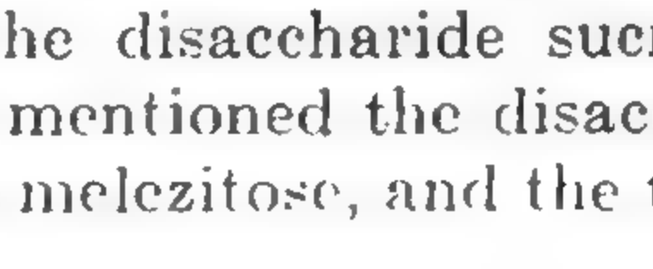
QUALITATIVE METHODS FOR THE IDENTIFICATION OF SUGARS

Probably no other class of organic compounds gives such a variety of reactions or forms so large a number of chemical derivatives as the sugars. Owing to the great extent of the field it will be possible to describe only a few of the more general tests and reactions.

In describing the various chemical tests, the sugars will be classified for convenience under two general groups: I. The reducing sugars. II. The non-reducing sugars. The reducing sugars are distinguished by the fact that they cause a marked precipitation of cuprous oxide when warmed with Fehling's alkaline copper solution, whereas the non-reducing sugars do not exhibit this property, or exhibit it to only a very slight extent after prolonged boiling. The reducing sugars constitute by far the larger group. The monosaccharides and many of their derivatives reduce Fehling's solution. Most of the disaccharides, including maltose, lactose, and the rarer sugars cellobiose, gentiobiose, melibiose, and turanose, also exhibit this property. The best-known non-reducing sugar is the disaccharide sucrose. Among other non-reducing sugars may be mentioned the disaccharide trehalose, the trisaccharides raffinose and melezitose, and the tetrasaccharide stachyose.

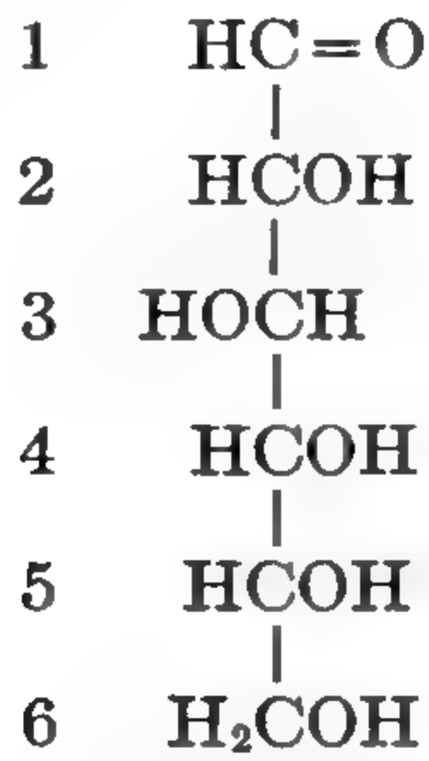
REACTIONS OF THE REDUCING SUGARS

The reducing sugars may react either as open-chain compounds whose characteristic chemical properties are due to a carbonyl-alcohol group  , or as ring compounds, with the common grouping

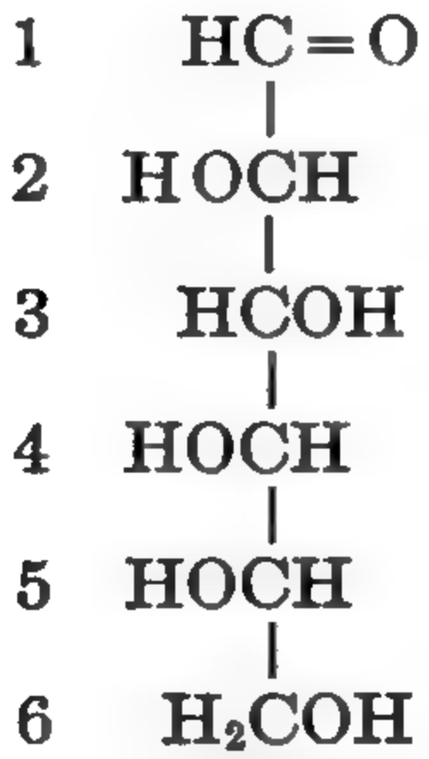


At this point a brief explanation may appropriately be given of the ways in which the three-dimensional arrangement of the atoms in the sugar molecule is projected onto a plane. The open-chain formulas

of *d*- and *l*-glucose, for example, may be written as shown in (I) and (II), respectively:

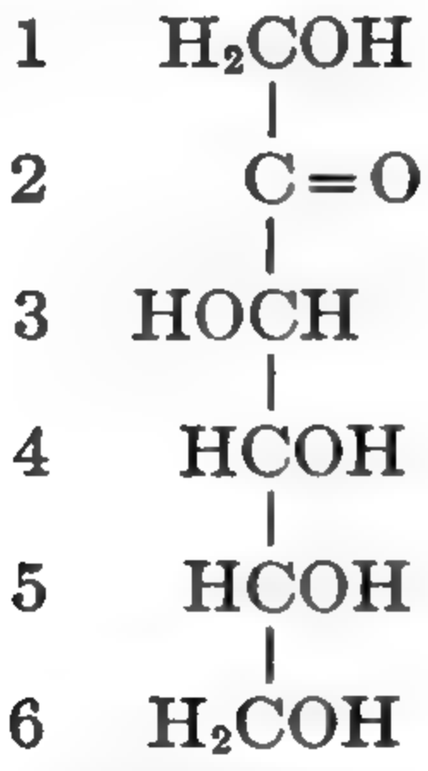


(I) *d*-Glucose

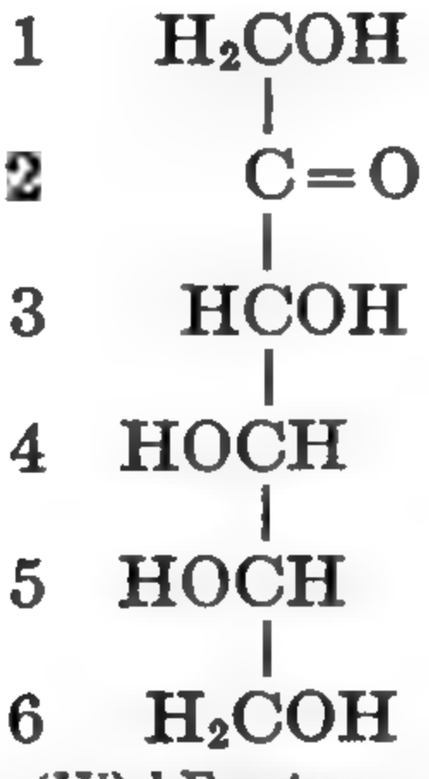


(II) *l*-Glucose

The carbon atom in the aldehyde group is designated as No. 1, and is placed on top. Analogously the formulas for the ketohexoses *d*- and *l*-fructose are written thus:



(III) *d*-Fructose



(IV) *l*-Fructose

The carbon atoms are numbered in the same sequence as in the corresponding glucose formulas, the carbonyl group being in position 2 instead of 1.

The formulas of the pentoses are written in the same manner as those of the corresponding hexoses, the H_2COH group in position 6 of the hexoses being shifted to position 5; in the tetroses it is shifted to position 4.

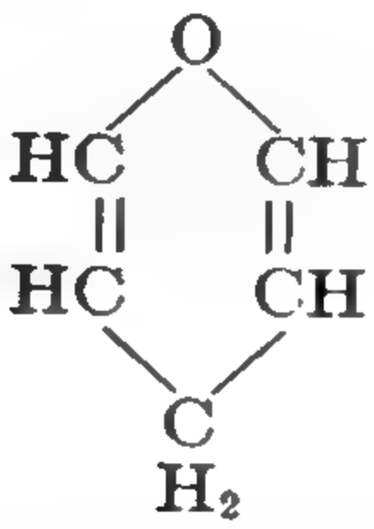
If it is desired to write a hexose chain formula in one line it is customary to place carbon atom 1 at the extreme right; for example:

$$CH_2OH-\underset{5}{C}HOH-\underset{4}{C}HOH-\underset{3}{C}HOH-\underset{2}{C}HOH-\underset{1}{C}HO$$

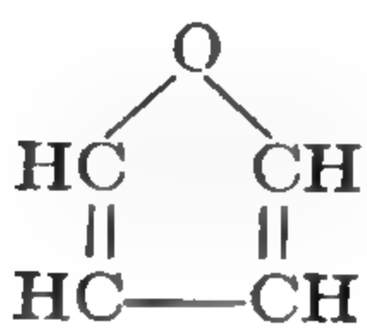
This formula may represent any of the 16 aldohexoses. The 8 ketohexoses with the keto group in position 2 are written similarly:

$$CH_2OH-\underset{5}{C}HOH-\underset{4}{C}HOH-\underset{3}{C}HOH-\underset{2}{C}O-\underset{1}{C}H_2OH$$

The ring form of the hexoses presents several possibilities. In the aldoses the oxygen bridge may connect carbon atom 1 with carbon atom 2 (methylene ring), 3 (ethylene ring), 4 (propylene ring), 5 (butylene ring), or 6 (amylene ring). In the ketoses carbon atom 2 may be connected with either 3, 4, 5, or 6. It has been found, however, that in most cases sugars and their derivatives have the amylene ring, connecting carbons 1 and 5 in the aldoses, and carbons 2 and 6 in the ketoses. This ring being similar to that in pyran (V), these sugars are termed pyranoses.



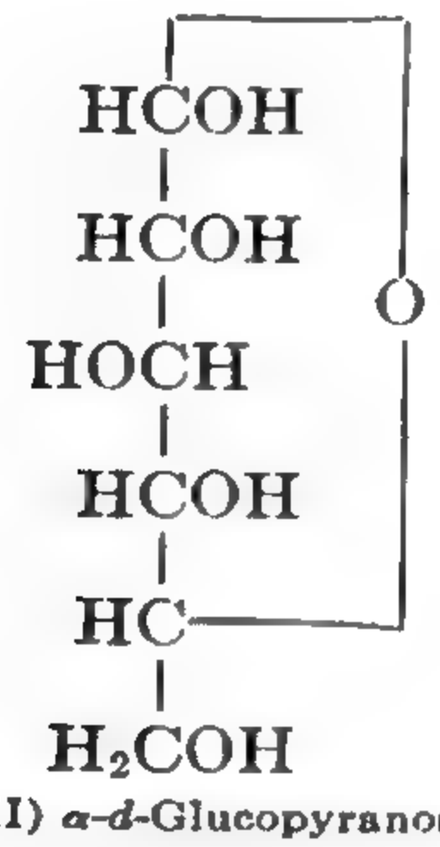
(V) Pyran



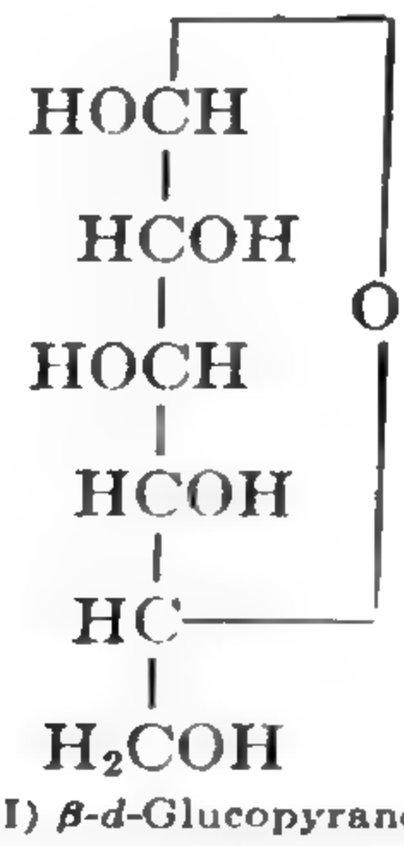
(VI) Furan

But a number of sugars or derivatives with a butylene ring are also known. Here the oxygen connects carbons 1 and 4 in the aldoses, and carbons 2 and 5 in the ketoses. These sugars are known as furanoses because the ring is similar to that in furan (VI).

The ring form of *d*-glucopyranose may be represented by the following two formulas:



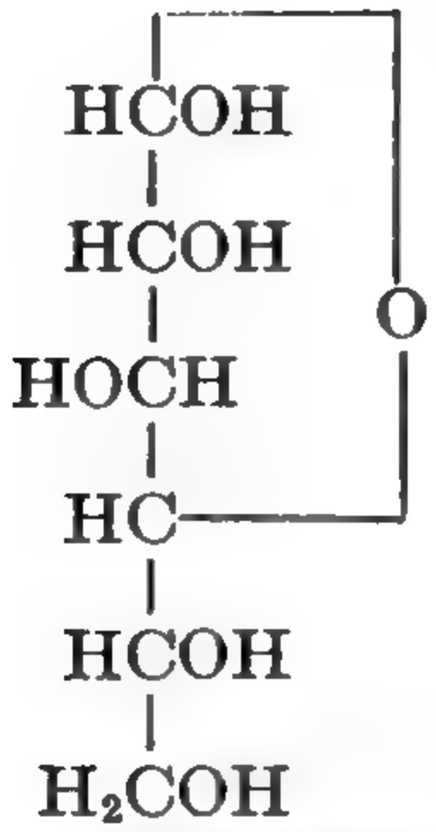
(VII) *α*-*d*-Glucopyranose



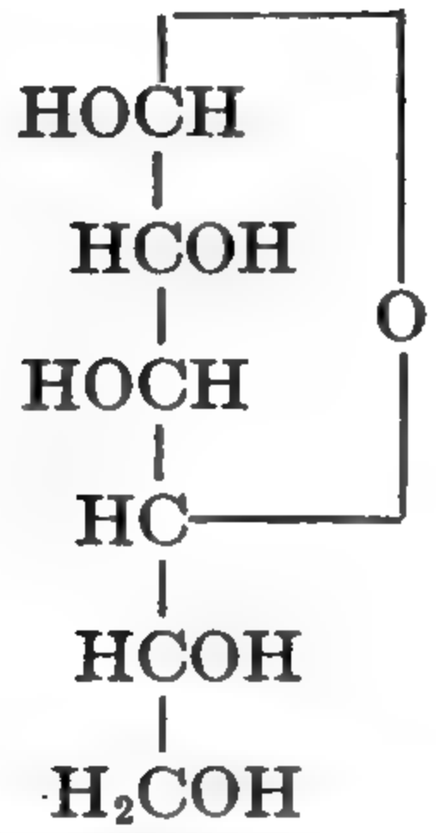
(VIII) *β*-*d*-Glucopyranose

These formulas show that the ring formation gives rise to a new asymmetric carbon atom, No. 1. The hydroxyl group on this carbon may be on the same side as the oxygen bridge (*cis* position), or on the opposite side (*trans* position). The first of these isomers (VII) is designated as *α*, and the second (VIII) as *β*. The corresponding glucofuranoses are

written as follows:



(IX) α-*d*-Glucofuranose

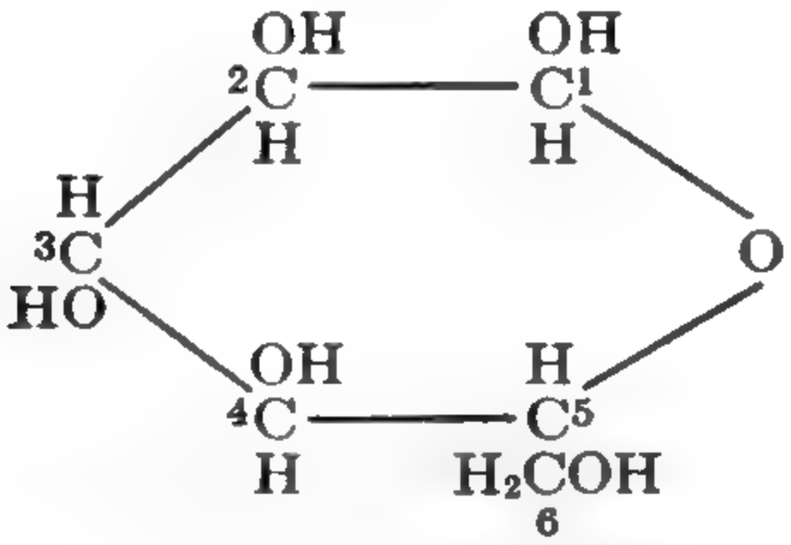


(X) β-*d*-Glucofuranose

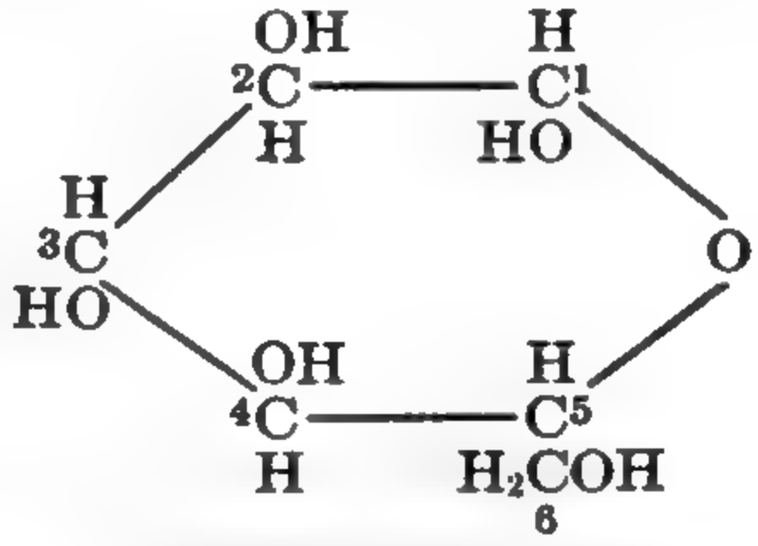
The formulas for the *l*-glucopyranoses and *l*-glucofuranoses are analogous to formulas VII, VIII, IX, and X, the oxygen bridge being placed on the left instead of the right.

In the ketose series the oxygen connects carbon atom 2 with either carbon 6 (pyranoses), or with carbon 5 (furanoses). It is interesting to note that ordinary *d*-fructose is a pyranose, but the fructose portion of the sucrose molecule is a furanose.

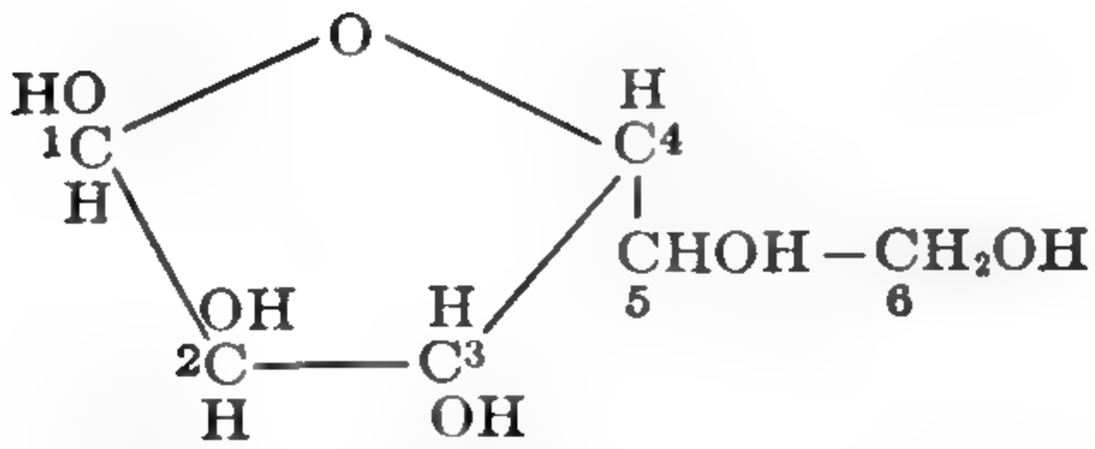
The spatial arrangement in the pyranoses and furanoses is shown better in the perspective formulas introduced by Haworth than in those given above:



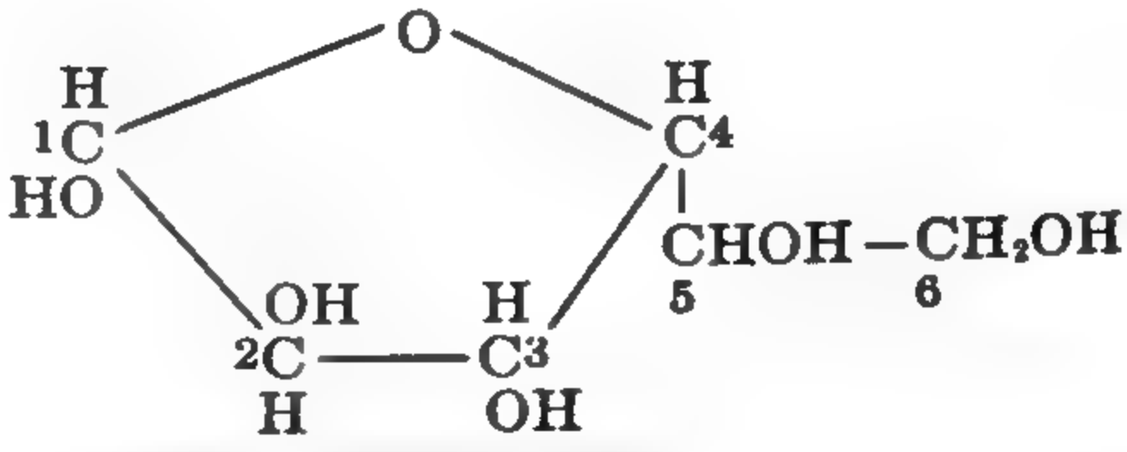
(XI) α-*d*-Glucopyranose



(XII) β-*d*-Glucopyranose



(XIII) α-*d*-Glucofuranose



(XIV) β-*d*-Glucofuranose

Although the reducing sugars are known to exist in solution almost entirely in the ring form, with only very small percentages of the alde-

hydo or keto form present, they nevertheless react in many tests like ordinary aldehydes or ketones. The chemist must, therefore, first of all, guard against deciding as to the presence of a sugar from a reaction which would also be given by formaldehyde, acetaldehyde, or acetone. A number of confirmatory tests must usually be applied before it can be stated definitely whether a sugar is or is not present.

The qualitative reactions for reducing sugars are divided for convenience into: I. General tests. II. Special tests. III. Individual tests. After it has been determined from general tests that a sugar is present, special tests must be applied in order to determine what classes or groups of sugars are present, whether hexoses or pentoses, aldoses or ketoses, monosaccharides or disaccharides. After the class or group of sugars has been ascertained, individual tests must be applied in order to determine what particular sugars are present.

General Tests for Reducing Sugars

Among the general tests which are sometimes given for sugars may be mentioned the familiar property which all carbohydrates have of giving off a characteristic sweetish odor upon heating over a flame in a closed tube. This odor, which is usually designated as caramel-like, is given off, however, by many polyatomic alcohols and acids (as by tartaric acid) so that the test is not characteristic of sugars alone. Among the decomposition products obtained by heating sugars in a closed tube may be mentioned (besides water and the gaseous products carbon dioxide and carbon monoxide) formic acid, acetic acid, acetone, furfural, and various products of an aldehyde nature. It is to the furfural and aldehyde products that the characteristic odor of burnt sugar is largely due.

The general tests for reducing sugars may be divided for convenience into four general groups of reactions.

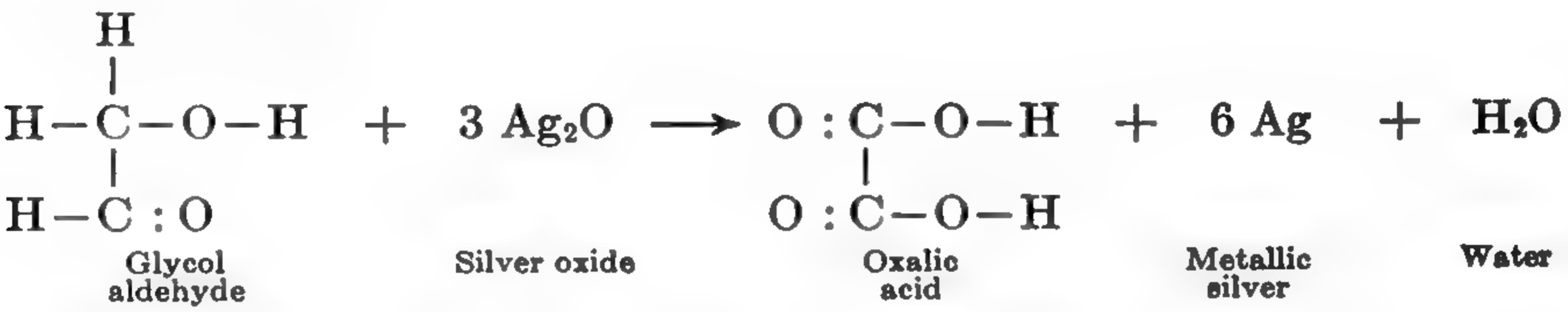
I. Reducing reactions with solutions of metallic salts or of organic compounds.
II. Color reactions with alkalies, acids, phenols, and other organic compounds.
III. Hydrazone and osazone reactions with phenylhydrazine and its substituted derivatives.
IV. Miscellaneous reactions.

I. Reducing Reactions of Sugars

The simple sugars and certain of the disaccharides, as maltose and lactose, have the property of reducing alkaline solutions of many metallic salts, such as those of copper, silver, mercury, and bismuth. This

reaction, which is common to most aldehydes, is due to the withdrawal of oxygen from the metallic base, the latter being precipitated either as a suboxide or in the metallic form. In the simplest case the aldehyde group is oxidized by the oxygen withdrawn from the metallic base to the acid carboxyl group, as indicated by the following general equation:

If an alcohol group is also present, further oxidation converts it into the carbonyl and then the carboxyl group as in the following reaction for glycol aldehyde:



This oxidation in the case of the higher monosaccharides is usually attended by a breaking down of the carbon chain as by the oxidation of glucose in ammoniacal silver solution:

$$C_6H_{12}O_6 + 9\,Ag_2O \longrightarrow 3(COOH)_2 + 18\,Ag + 3\,H_2O$$

The reaction between sugars and alkaline salts of metals, as ordinarily carried out, gives rise to a number of monobasic and dibasic acids (formic, oxalic, etc.) in varying proportions according to the conditions of the experiment. It is not possible, therefore, to express the reaction by chemical equations except in a very general way.

The most common of the alkaline salt solutions employed in testing sugars are those of copper. The sulfate and acetate of copper are the salts most generally used, and sugar literature is filled with descriptions of modifications for making the test. Only a few of these will be described.

Fehling's Copper Solution. This is the most common chemical reagent employed in testing sugars. As ordinarily prepared the reagent consists of two solutions: solution *A* containing 34.64 g. crystallized copper sulfate to 500 ml., and solution *B* containing 173 g. Rochelle salts and 50 g. sodium hydroxide to 500 ml. The solutions are the same as those used in quantitative analysis and are to be kept separate until just before using. By mixing 5 ml. each of solutions *A* and *B* in a test tube, adding a few milliliters of the solution to be examined, and heating to boiling for 2 minutes, a brick-colored precipitate of cuprous

oxide, Cu_2O, will form, if reducing sugars are present, the intensity of coloration and amount of precipitate being proportional to the amount of sugar present. The test is sensitive to about 0.01 mg. of glucose to 1 ml.

Products Obtained by Heating Reducing Sugars with Fehling's Solutions. The chemical reactions which take place in the oxidation of sugars by means of Fehling's solution are exceedingly complex. Nef,[1] who made the first systematic studies in this field, found that in *l*-arabinose the oxidation proceeds along three separate lines.

I. From 10 to 25 per cent of sugar are oxidized to form pentonic acids.

$$C_5H_{10}O_5 + O \longrightarrow C_5H_{10}O_6$$

II. From 35 to 45 per cent of sugar are oxidized to form formic and trioxybutyric acids.

$$C_5H_{10}O_5 + 2\,O \longrightarrow HCOOH + C_4H_8O_5$$

III. From 30 to 38 per cent of sugar are oxidized to form formic and glycolic acids.

$$C_5H_{10}O_5 + 3\,O \longrightarrow HCOOH + 2\,C_2H_4O_3$$

With the hexose sugars, *d*-glucose, *d*-mannose, and *d*-fructose, Nef obtained analogous reactions with formation of carbonic, formic, glycolic, glyceric, trioxybutyric, and hexonic acids. The formation of all these products has been explained on the basis of the fact that the reducing sugars are not stable in alkaline solution, but undergo molecular rearrangements and are partly broken up into compounds with shorter carbon chains. The cupric oxide in Fehling's solution oxidizes the reaction products, with the formation of the acids named above. The primary effect of the alkali is described in greater detail on p. 653.

In testing solutions containing much foreign organic matter such as urine, the reaction with Fehling's solution may be interfered with. Uric acid, creatine, creatinine, albumin, peptones, and other substances may either check the precipitation of cuprous oxide, when reducing sugars are present, or sometimes cause a precipitate of copper in the complete absence of sugars. Solutions containing xanthine bases, such as low-grade molasses or distillery waste, when heated with Fehling's solution may precipitate greenish yellow copper compounds, which may be mistaken for cuprous oxide. In all such cases the impure solution should be clarified with a little normal acetate of lead and filtered; any

[1] *Ann.*, 357, 214–312 (1907).

excess of lead is removed from the filtrate with sodium carbonate and the clear solution tested with Fehling's reagent in the usual way. When amino acids and their derivatives are present, mercuric nitrate or acetate is preferable to lead salts. Filtering the impure solution through animal charcoal is also of advantage when foreign coloring matter masks the reaction.

Richtmyer and Hudson[2] have made the important observation that the reducing effect of certain sugars belonging to the *d* series upon copper reagents prepared with *l*-tartrates, instead of the usual *d*-tartrates employed in Fehling's solution, is different from that of the corresponding sugars of the *l* series. Thus the reducing power of *l*-arabinose upon the *l*-tartrate reagent is only 87 per cent of that on the *d*-tartrate reagent, while that of *d*-arabinose is 115 per cent of that on the *d*-tartrate reagent. In other cases, as for example *d*-glucose, the reducing effect on the two tartrate reagents is practically the same. This behavior toward optically active copper reagents may be utilized for the identification of sugars.

Benedict's Copper Solution. Instead of the sulfate-tartrate, solutions of other copper salts have been employed in testing for sugars. Benedict's reagent[3] is prepared by dissolving 173 g. of sodium citrate and 100 g. of anhydrous sodium carbonate in about 800 ml. of water, and 17.3 g. of crystallized copper sulfate in about 100 ml. of water. The two solutions are mixed and diluted to 1 liter. This solution is not reduced by creatine, creatinine, uric acid, or similar substances, and is especially adapted for the testing of urine.

Barfoed's Copper Solution. Barfoed[4] has prepared a solution containing 1 part crystallized neutral copper acetate in 15 parts of water; 5 ml. of 38 per cent acetic acid is added to 200 ml. of the copper acetate solution before use. On boiling the solution a basic acetate of copper is formed, the liberated cupric oxide being reduced in the presence of monosaccharides. Barfoed's reagent is not reduced to any great extent by the disaccharides, lactose and maltose, and is, therefore, of value in distinguishing these sugars from monosaccharides.

Soldaini's Copper Solution. Carbonate of copper solution has also been used in testing for sugars. Soldaini[5] has prepared a solution containing 15 g. precipitated copper carbonate, $CuCO_3$, and 416 g. potassium bicarbonate, $KHCO_3$, dissolved to 1400 ml. Instead of starting

[2] *J. Am. Chem. Soc.*, **58**, 2540 (1936).

[3] *J. Biol. Chem.*, **5**, 485 (1909); see also Samson, *J. Am. Chem. Soc.*, **61**, 2389 (1939).

[4] *Z. anal. Chem.*, 12, 27 (1873).

[5] *Z. Ver. deut. Zucker-Ind.*, 39, 933 (1889); **40**, 792 (1890).

with copper carbonate, copper sulfate may be used, a solution of which is added to the $KHCO_3$ solution, the precipitate of $CuCO_3$ first formed being dissolved in the excess of bicarbonate. A solution containing 3.464 g. copper sulfate and 297 g. potassium bicarbonate to 1000 ml. is especially adapted for detecting small amounts of reducing sugars.

Among other copper solutions recommended for testing sugars may be mentioned copper ammonium tartrate and ammoniacal copper sulfate or acetate. None of these preparations, however, has been found to equal Fehling's reagent for general usefulness in practical sugar analysis.

Tollens's Silver Solution. The most sensitive of metallic-salt solutions for detecting sugars is ammoniacal silver solution, first employed by Tollens[6] and hence usually known as Tollens's reagent. This is prepared by dissolving 1 part silver nitrate in 10 parts of water; a second solution is then made containing 1 part sodium hydroxide in 10 parts of water. Before making the test equal parts of the two solutions are mixed and then ammonia added drop by drop until the precipitate of silver oxide is completely dissolved. A solution containing 1 part of glucose in 1000 parts of water will cause a strong reduction of Tollens's reagent in the cold, a mirror of silver being deposited within 15 minutes. A solution containing 1 part glucose to 100,000 parts of water will also produce a perceptible reduction in the cold, but the solution must stand 1 to 2 days. The reduction takes place more rapidly upon warming. but warming or heating the solution is to be avoided owing to the danger of forming explosive silver compounds. For the same reason the reagent should be prepared only just before using. Tests should be carried out in the dark, and solutions containing the reagent should not be kept for any length of time.

The sensitivity of the test can be further increased by the use of Feigl's procedure.[7] A drop of the sugar solution is placed on a piece of filter paper impregnated with silver nitrate solution. A drop of alkali is placed next to the drop of sugar solution, and after a short while the filter paper is dipped in ammonia; this dissolves the silver oxide, and a black spot of metallic silver remains. As little as 2 parts per 1,000,000 of reducing sugar can be detected in this way.

Tollens's silver reagent is also reduced by all aldehyde substances; it is affected not only by the sugars which reduce Fehling's solution but also by sucrose, raffinose, and all other soluble carbohydrates. Even the alcohol derivatives of the sugars produce reduction, glycerol, for example, causing the formation of a silver mirror. The readiness

[6] *Ber.*, **15**, 1635 (1882); **16**, 921 (1883).
[7] *Intern. Sugar J.*, **41**, 147 (1939).

with which ammoniacal silver solution is reduced by soluble organic non-sugars has proved a serious objection against the use of this reagent in ordinary analytical work.

Knapp's Mercury Solution. A third reagent which has been used for testing sugars is Knapp's[8] alkaline mercuric cyanide solution. This contains 10 g. of mercuric cyanide dissolved in 100 ml. sodium hydroxide solution of 1.145 specific gravity. Similar alkaline solutions have been prepared by Sachsse[9] from mercuric iodide and by Bauer[10] from mercuric chloride. These solutions are reduced upon warming with sugar solutions giving grayish deposits of metallic mercury. The mercury solutions have the same objection, however, as those of silver in being reduced by different organic non-sugars, such as creatine, creatinine, and glycerol, and even under certain conditions by alcohol. Alkaline solutions of mercury salts are, therefore, of but little value in detecting sugar in urine and other liquids rich in organic non-sugars.

Nylander's Bismuth Solution. A fourth reagent, which has been used considerably for detecting reducing sugars in urine, is an alkaline solution of bismuth subnitrate, known as Nylander's[11] (or Almén's) reagent. This solution as prepared by Nylander is made by dissolving 2 g. of bismuth subnitrate and 4 g. of Rochelle salts in 100 g. of 8 per cent sodium hydroxide solution. After standing for a few days the solution is filtered through glass wool and the clear filtrate preserved in a stoppered bottle. The solution will keep indefinitely. When Nylander's reagent is heated with a solution containing reducing sugars a precipitate of dark metallic bismuth is produced. Heating with one-tenth its volume of 0.01 per cent glucose solution will cause a perceptible darkening. In testing urine 1 ml. of the reagent and 10 ml. of urine are heated in a test tube 2 to 5 minutes over the flame; after standing for 5 minutes the solution is examined for the appearance of a dark-colored sediment.

Nylander's reagent, however, is open to the same objections noted for the alkaline silver and mercury solutions. The presence of albumin, nuclein, glucuronic acid, and other organic non-sugars in urine will also cause a precipitation of bismuth, even when glucose is completely absent. While the failure of a precipitate with Nylander's reagent may indicate the absence of reducing sugars, the occurrence of a precipitate may be said to indicate the presence of sugar only when reducing non-sugars are proved to be absent.

[8] *Z. anal. Chem.*, 9, 395 (1870).
[9] *Z. Ver. deut. Zucker-Ind.*, 26, 872 (1876).
[10] *Landw. Vers.-Stat.*, 36, 304 (1881).
[11] *Z. physiol. Chem.*, 8, 175 (1883/84).

Miscellaneous Solutions of Metallic Salts. Of other alkaline solutions of metallic salts proposed for sugar testing may be mentioned alkaline nickel sulfate and tartaric acid which gives a dark-red precipitate of nickel suboxide in the presence of reducing sugars; alkaline ferric chloride and sodium tartrate which gives a brown-colored precipitate on heating with reducing sugars; cobaltous nitrate and alkali which gives a blue color, changing to light green, with glucose; and gold chloride and alkali, which is reduced to purple or blue colloidal gold. None of these reagents, however, or any of the other alkaline solutions of metallic salts previously mentioned, has been found to equal Fehling's copper reagent for all-around usefulness and reliability.

Effect of Reducing Sugars on Molybdates. Another reaction of reducing sugars, widely used in the sugar industry to detect traces of sucrose, is their reducing effect on molybdates in acid solution. This reaction was first described by Cotton, who used it for detecting sucrose in milk, as it was found that lactose acts only very slowly. Pinoff found that, if glacial acetic acid is used instead of a mineral acid, fructose reacts much more quickly than aldoses. Pinoff and Gude[12] give the following procedure for making this test. To 10 ml. of the sugar solution add 10 ml. of 4 per cent ammonium molybdate solution and 0.2 ml. of glacial acetic acid. Heat for 3 minutes in live steam. A blue color develops rapidly with small quantities of fructose, but much larger amounts of glucose are required to give the test.

According to Dorfmüller[13] ammonium molybdate may be substituted for α-naphthol to detect sugar in factory condensates and sweet waters. Ten drops of 12 per cent hydrochloric acid and 20 drops of a 20 per cent solution of ammonium molybdate are added to 0.5 ml. of the sample. Upon boiling the blue color develops more or less rapidly, depending on the amount of sugar present.

Matthews[14] has modified the test so that it may be used for approximate quantitative determinations. Five milliliters of the sample is placed in a clean test tube, 3 drops of concentrated hydrochloric acid and 3 ml. of a 4 per cent ammonium molybdate solution are added, and the tube is placed in a boiling-water bath for exactly 6 minutes. Standards are prepared by diluting a solution containing 1 g. of sucrose per liter to convenient concentrations and treating these solutions exactly as described for the test. Permanent standards may be made by diluting blue-black ink to match the freshly prepared primary standards. For concentrations below 0.0125 per cent sucrose, diluted Feh-

[12] *Chem. Ztg.*, 38, 625 (1914).
[13] *Deut. Zuckerind.*, 44, 574 (1919).
[14] *Maryland Acad. Sci. Bull.* 7, No. 3, p. 35, 1928.

ling's solution must be used instead of ink to get a perfect color match. These secondary standards keep for 6 months. The conditions of the test must be strictly adhered to because even slight variations in detail may cause large errors.

Selenious Acid Test for Reducing Sugars. Selenious acid is reduced to red selenium by reducing sugars, as shown by Reif.[15] About 0.1 g. of the sugar is dissolved in 15 ml. of water, 15 drops of a solution of 0.5 g. selenious acid in 100 ml. of concentrated sulfuric acid is added, and the mixture is heated in a boiling-water bath for 20 to 25 minutes. A red precipitate indicates reducing sugars. Fructose gives about 20 times as much precipitate as glucose or other aldoses. The test may be used for approximate quantitative estimation of the various reducing sugars.

Reduction of Nitro Compounds. Among the reagents used for detecting sugars on the basis of their reducing effect are also included a number of organic compounds.

Picric Acid Test. Picric acid, $C_6H_2(NO_2)_3OH$, is reduced to amido dinitrophenol, $C_6H_2(NO_2)_2NH_2OH$, which has a deep red color. The reaction, first observed by Braun,[16] has been widely employed for detecting sugar in boiler feedwater. The water sample is boiled with 2 to 3 drops of hydrochloric acid to invert the sucrose; it is then made alkaline with sodium hydroxide, and 2 to 3 drops of an alcoholic solution of picric acid is added. The reaction is not so sensitive as the α-naphthol test but gives positive results at a sugar concentration of 1 : 5000. Several quantitative methods based on this reaction are discussed in Chapter XIV.

o-Dinitrobenzene Test. This is carried out as follows, according to Bose:[17] One drop of a 1 per cent solution of the reagent in alcohol is mixed with 2 ml. of a 25 per cent solution of sodium carbonate in a test tube, and 1 ml. of the sugar solution is added. Upon heating the mixture for 15 to 20 seconds a deep violet color is obtained which gradually fades. If the solution is made acid the color disappears, but immediate addition of alkali brings it back again. The reaction is very sensitive, permitting the detection of 6 parts per 1,000,000 of glucose, fructose, galactose, mannose, lactose, or rhamnose, or 3 parts per 1,000,000 of arabinose. Non-reducing sugars do not give the test.

Other nitro compounds, such as *m*-dinitrobenzene, *m*- or *p*-nitrobenzaldehyde, and dinitrosalicylic acid, give similar reactions.

An interesting example of reactions belonging to this class is the re-

[15] *Z. Untersuch. Lebensm.*, **71**, 439 (1936); **73**, 20 (1937).
[16] *Z. anal. Chem.*, **4**, 185 (1865).
[17] *Z. anal. Chem.*, **87**, 110 (1932).

duction, followed by condensation, of *o*-nitrophenylpropiolic acid, $NO_2—C_6H_4—C\equiv C—COOH$, to indigo, by glucose and alkali.[18] It is one of the synthetic methods proposed for the commercial production of that dyestuff. By further treatment with glucose and alkali the indigo is reduced to indigo white.

Reduction Tests with Dyestuffs. A dilute alkaline solution of methylene blue (1 g. per liter) is rapidly reduced and decolorized by fructose, and more slowly by glucose, upon heating.[19] This reaction is utilized in several quantitative methods (see Chapter XIV). Safranine solution changes in color from red to yellow when heated in the presence of reducing sugars and alkali.[20] Both dyestuffs have been advocated instead of α-naphthol for detecting sugar in waste and condenser waters, after inversion with acid. Herzfeld[21] recommended methylene blue to test raw beet sugars for traces of invert sugar.

II. COLOR REACTIONS OF SUGARS WITH ALKALIES, ACIDS, PHENOLS, ETC.

As a second general reaction of reducing sugars may be mentioned certain color effects which nearly all soluble carbohydrates give when brought into contact with different reagents. The reagents employed may be divided into three groups:

I. Alkalies.
II. Concentrated mineral acids.
III. Phenols and other organic compounds.

Color Reactions of Sugars with Alkalies. All reducing sugars have the property of coloring solutions of the alkalies and alkaline earths yellow, the application of heat turning the color a dark brown. This reaction is common to all aldehydes. The exact nature of the coloring matter formed by the action of alkalies upon sugars in solution is not understood. Considerable oxygen is absorbed from the air during the reaction, and various products of an acid nature are among the substances formed.

Products Obtained by Heating Reducing Sugars with Alkali. Lactic acid is produced in considerable amount by the action of alkalies upon many reducing sugars such as xylose, arabinose, glucose, and fructose. The presence of calcium lactate in certain sugar-cane molasses is explained by the action of an excess of lime during clarification upon the

[18] Ihl, *Chem. Ztg.*, 12, 25 (1888).
[19] Hall, E. and H. Armstrong, Keeble and Russell, *J. Soc. Chem. Ind.*, 35, 648 (1916).
[20] Phelan, *Proc. Second Annual Conference, Queensland Soc. of Sugar Technologists*, p. 62, 1931.
[21] *Deut. Zuckerind.*, 13, 234 (1888).

reducing sugars of the juice. Formic, acetic, and oxalic acids have also been found among the products resulting from the action of alkalies upon sugars in solution. Certain phenol bodies such as pyrocatechol and protocatechuic acid have also been detected among the oxidation products of sugars resulting from treatment with alkalies.

Nef[22] has studied the action of $\frac{1}{8}$ normal sodium hydroxide upon different sugars and obtained in case of *d*-glucose, *d*-mannose, and *d*-fructose a yield of 40 to 45 per cent *d,l*-lactic, from 10 to 15 per cent *d,l*-1-hydroxybutyrolactone, about 25 per cent of saccharin, metasaccharin, and isosaccharin, and a small quantity of tarry decomposition products.

Nef[23] also developed a theory explaining the formation of these various reaction products; his theory has been tested, and revised in certain respects by Evans and coworkers,[24] as well as by other investigators. It is based on the suggestion, originally advanced by Wohl and Neuberg, that the sugars may react as enediols. The alkali causes a splitting of the ring, the aldehyde or keto form of the sugar results, and the enediols are formed by a migration of hydrogen atoms or ions. *d*-Glucose, for instance, may form the following three isomers:

$$CH_2OH-CHOH-CHOH-CHOH-COH = CHOH \quad \text{(1,2 enediol)}$$
$$CH_2OH-CHOH-CHOH-COH = COH-CH_2OH \quad \text{(2,3 enediol)}$$
$$CH_2OH-CHOH-COH = COH-CHOH-CH_2OH \quad \text{(3,4 enediol)}$$

Increase in the alkali concentration shifts the equilibrium from the 1,2 enediol toward the 3,4 enediol.

These reactive substances undergo further transformation in various ways:

Splitting of enediols. When the enediols split at the double bond the 1,2 enediol gives the methylene enol of a pentose and formaldehyde, the 2,3 enediol those of a tetrose and glycolic aldehyde, and the 3,4 enediol two molecules of glyceric aldehyde methylene enol. The pentoses and tetroses may split further through repeated enediol formation. In the presence of air or oxidizing agents the cleavage products are converted into acids. The glyceric aldehyde in the presence of alkali yields pyruvic aldehyde, and this goes over into lactic acid by intermolecular rearrangement. The pyruvic aldehyde may also split into acetaldehyde and carbon monoxide which appears as formic acid. Acetic acid is formed by oxidation of the acetaldehyde.

[22] *Ann.*, **376**, 1–119 (1910).

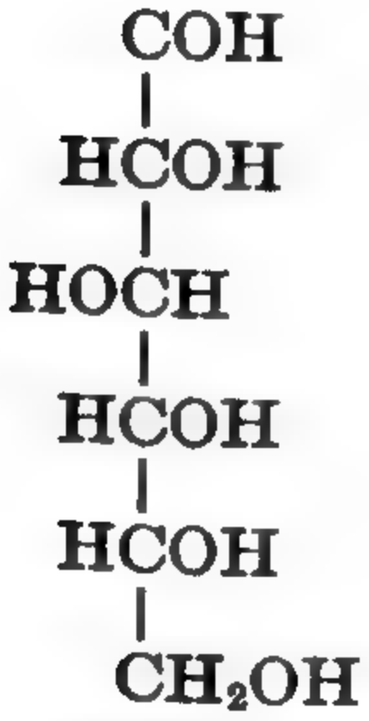
[23] *Ann.*, **403**, 204–383 (1914).

[24] *J. Am. Chem. Soc.*, **47**, 3085, 3098, 3102 (1925); **48**, 2665, 2678, 2703 (1926); **50**, 486, 1496, 2543 (1928); **52**, 294, 3680, 4065 (1930); **53**, 4384 (1931); **54**, 698 (1932).

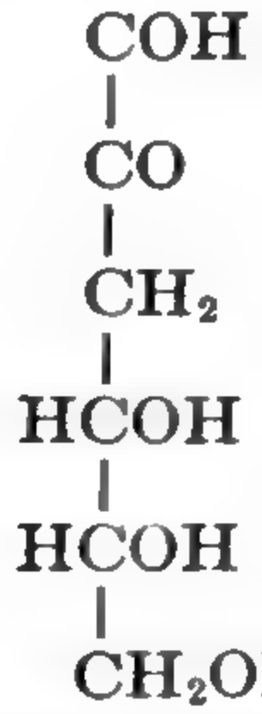
Mutual transformation of monoses. By a shifting of the hydrogen atoms the glucose 1,2 enediol may change into either fructose or mannose. The 2,3 enediol may change to a 3-ketohexose, and this again into the 3,4 enediol. This accounts for the mutual transformations of glucose, fructose, and mannose, and similar transformations in other monose series, first observed by de Bruyn and van Ekenstein upon treatment with dilute alkali. Boiling with pyridine has the same effect as treatment with weak alkali.

Kuzin[25] has shown that sodium hydroxide and calcium hydroxide act differently in the enolization of *d*-glucose. Calcium hydroxide produces an enol without splitting the ring; hence an appreciable amount of mannose is formed, but no fructose. Sodium hydroxide, on the other hand, splits the ring, and in the rearrangement considerable fructose appears, but little mannose.

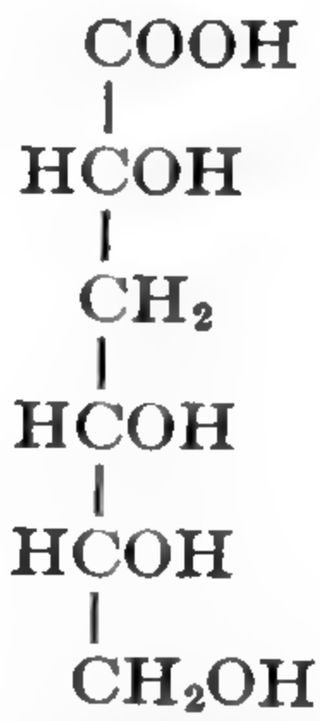
Saccharinic Acids. Nef explained the production of these acids by the intermediate formation of desoxyosones which are then converted into the corresponding saccharinic acids by the benzilic acid rearrangement. According to this theory the aldoses yield the corresponding metasaccharinic acids, as shown by the following example of *d*-glucose:



d-Glucose



d-Glucose 3-desoxy-1,2-osone



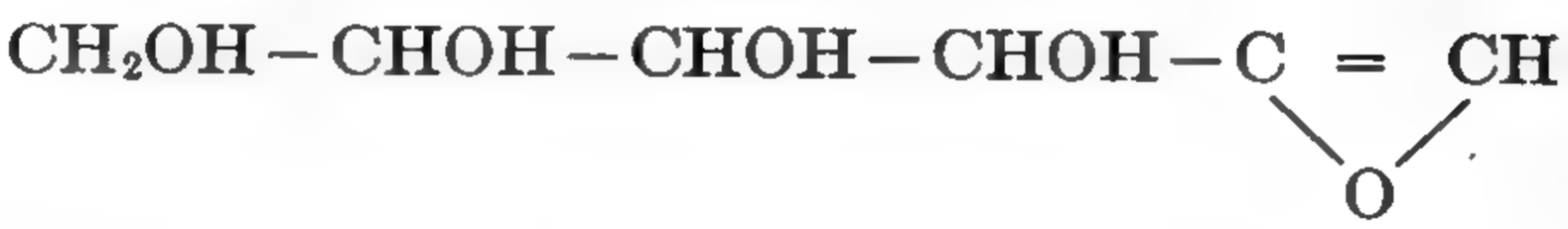
d-Gluco-metasaccharinic acid

Similarly, fructose and 2-ketohexoses in general give isosaccharinic acids, and 3-ketohexoses yield normal and parasaccharinic acids. Each of the saccharinic acids exists in two stereoisomeric forms, termed α and β.

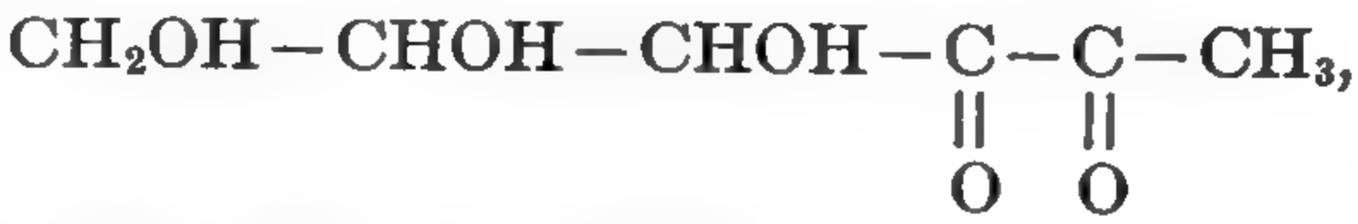
Evans and Benoy[26] have furnished an explanation of the formation of the desoxyosones also on the basis of the dienol theory. For instance, the 1,2 enediol $CH_2OH—CHOH—CHOH—CHOH—COH{=}CHOH$

[25] *Ber.*, **69B**, 1041 (1936).
[26] *J. Am. Chem. Soc.*, **48**, 2675 (1926).

splits off water and by ring formation gives



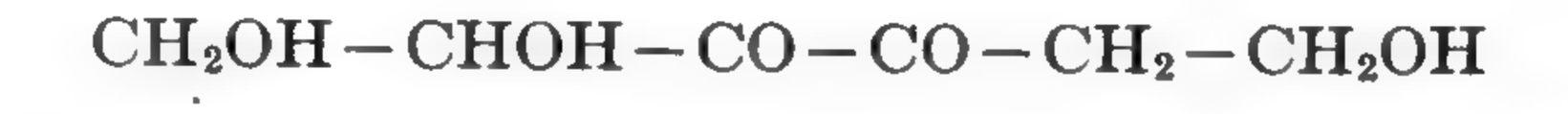
This compound rearranges to the desoxyosone



and then to normal saccharinic acid,

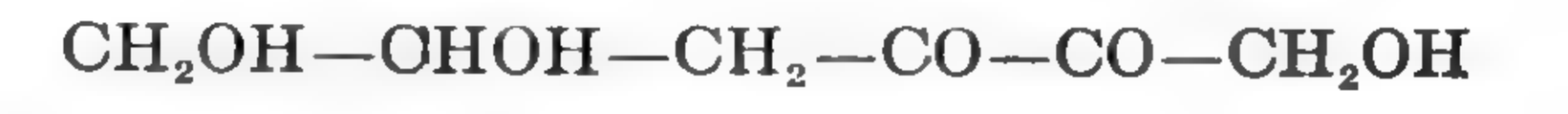
Similarly, the 2,3 enediol first gives

This compound may give rise to two desoxyosones:

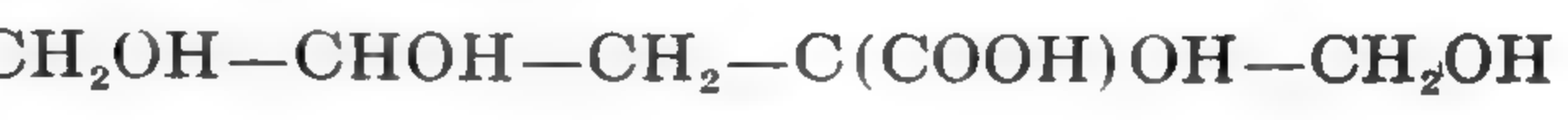
and 

The former changes to metasaccharinic acid, as shown above, and the latter to parasaccharinic acid,

The 3,4 enediol is transformed to the desoxyosone



and this to isosaccharinic acid,



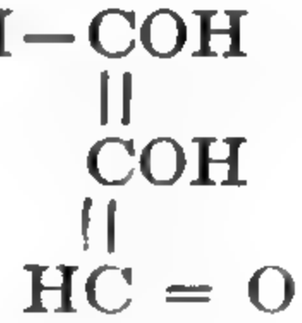
Evans holds, contrary to Nef, that in this way any of the saccharinic acids may be formed from any of the corresponding hexoses.

The saccharinic acids have not been obtained in the free state, because they split off water and are changed to the γ-lactones which usually crystallize readily.

Effect of Alkali on Disaccharides. The effect of alkali on the reducing disaccharides is much more complex than in the case of the monoses. Evans has found that maltose forms a 1,2 and a 2,3 enediol in the non-glucosidic portion of the molecule, but not a 3,4 enediol, because this is blocked by the glucosidic linkage at carbon atom 4. The 1,2 enediol

may split into hydroxymethylene and 3-glucosidoarabinose, and this again into hydroxymethylene and 2-glucosidoerythrose. The 2,3 enediol gives 2-glucosidoerythrose and glycolaldehyde. Besides the cleavage of the enediols there is also a slow hydrolysis at the glucoside linkage under the influence of alkali. Among the final products there are found, as with the monoses, lactic acid, pyruvic aldehyde, and formic and acetic acids. Lactose and cellobiose, being 4-glucosides, behave similarly as maltose. But melibiose and gentiobiose, which are 6-glucosides, form four enediols, 1–2, 2–3, 3–4, and 4–5, which may split in different ways. For a detailed account of the many possible reactions due to these rearrangements the original papers of Evans and collaborators should be consulted.

Glucic Acid. Another interesting product of the effect of alkali solutions on glucose at high temperature is the glucic acid of Winter.[27] Both the acid and its salts are strong reducing agents and are very unstable. The free acid readily oxidizes to formic and oxalic acids, and the calcium salt rapidly absorbs oxygen from the air, with decomposition and evolution of heat. Nelson and Browne[28] considered glucic acid to be the enolic form of the semialdehyde of malonic acid, CHOH=CH—COOH. Later Nelson[29] showed that it is identical with the "reductone" of Euler and Martius,[30] which is oxymalonic dialdehyde of the formula



According to Browne[31] the calcium salt is largely responsible for the spontaneous decomposition of molasses resulting from strongly limed cane juice. The calcium salt of glucic acid is prepared by mixing 400 g. crystallized glucose, 5000 ml. water, and 100 g. CaO after slaking in a stream of illuminating gas to prevent oxidation. On heating the solution to 67° C. and then cooling, the calcium salt settles as a volumi-
nous precipitate. This is filtered off, washed, exposure to air being avoided, and then dried in vacuum over concentrated sulfuric acid. The crystalline glucic acid is obtained by decomposing the calcium salt

[27] *Z. Ver. Rübenzuckerind.*, 44, 1049 (1894).
[28] *J. Am. Chem. Soc.*, 51, 830 (1929).
[29] Browne, *J. Assoc. Official Agr. Chem.*, 20, 394 (1937).
[30] *Ann.*, 505, 73 (1933).
[31] *Ind. Eng. Chem.*, 21, 600 (1929).

with the equivalent amount of dilute sulfuric acid, extracting with ether, and allowing the ethereal solution to evaporate.

Color Reactions of Sugars with Mineral Acids. Treatment of solutions of sugars and carbohydrates with concentrated mineral acids gives rise to a number of decomposition products, the color of which frequently throws some light upon the nature of the sugars present. The acids most commonly used for this purpose are sulfuric and hydrochloric. The color generated will depend partly upon the kind of sugar, partly upon the strength of acid used, and partly upon the temperature of the reaction.

Products Obtained by Heating Sugars with Acids. The darkening produced in all sugar solutions upon warming with concentrated sulfuric or hydrochloric acid is due largely to the formation of insoluble so-called "humus" substances of relatively high carbon content (C = 62 to 67 per cent and H = 3.5 to 4.5 per cent), the percentage of carbon and depth of color increasing with the strength of acid used. Attempts have been made to classify the humus substances formed by the action of acid upon sugars into ulmin and humin and ulmic and humic acids, to which various formulas have been assigned by different authorities. The constitution of the humus substances has not been definitely settled, however, and until considerably more work has been done the formulas of these must remain more or less a matter of conjecture.

In addition to the insoluble humus substances a number of soluble and volatile products are formed by the action of sulfuric and hydrochloric acids upon sugars. Among such products may be mentioned formic acid, levulinic acid, furfural, methylfurfural, hydroxymethylfurfural and a number of dextrinlike condensation or reversion products of high specific rotation. The nature and amount of these various products depend largely upon the kind of sugar, and a number of methods of group distinction are based upon the separation of characteristic decomposition products. Further reference will be made to these under the special reactions.

The ketoses are much more easily decomposed by strong mineral acids than the aldoses, and their solutions give rise to color reactions with correspondingly greater facility. This offers one means of distinguishing between a ketose and an aldose or of detecting a ketose sugar in the presence of an aldose. If a cold sugar solution is treated in a test tube with a few millimeters of concentrated sulfuric acid, allowing the latter to flow down the walls of the tube to the bottom without shaking, a rose-colored to brown ring will quickly form at the junction of the acid and sugar solution if fructose, sucrose, or a sugar

containing the ketone group is present; with glucose, lactose, maltose, and the aldoses in general the coloration will develop only slowly or not at all.

According to Colin and Ruppol[32] hydrochloric acid gives a more sensitive color reaction for ketoses than sulfuric acid. A wad of asbestos in the bottom of a test tube is moistened with fuming hydrochloric acid, a few grains of the sugar placed on top of the wad, and the tube closed with a stopper. Fructose or sorbose are colored violet in a few minutes, and the color gradually darkens. Carbohydrates which contain fructose as a cleavage product, such as sucrose, raffinose, and inulin, give the same reaction, only a little more slowly. Aldoses develop only a light yellow color in 2 to 3 hours. The test may also be carried out by placing the solid sugar in ether or chloroform saturated with hydrogen chloride gas.

Color Reactions of Sugars with Phenols. The most distinctive color reactions of the sugars are those obtained by treatment with different phenols in the presence of concentrated hydrochloric or sulfuric acid. The development of a color in this case is due to the formation of condensation products between the phenol derivatives and the decomposition products obtained from the sugar, particularly furfural and its derivatives. α-Naphthol, thymol, resorcinol, orcinol, naphthoresorcinol, phloroglucinol, cresol, and lysol are among the more important phenolic compounds used for making color reactions with sugars. A typical example of the condensation products between furfural and the phenols is di-α-naphtholfurylmethane, $C_{25}H_{18}O_3$, which has been obtained by Bredereck[33] from α-naphthol and furfural. It is colorless, but dissolves in concentrated sulfuric acid with a deep violet color.

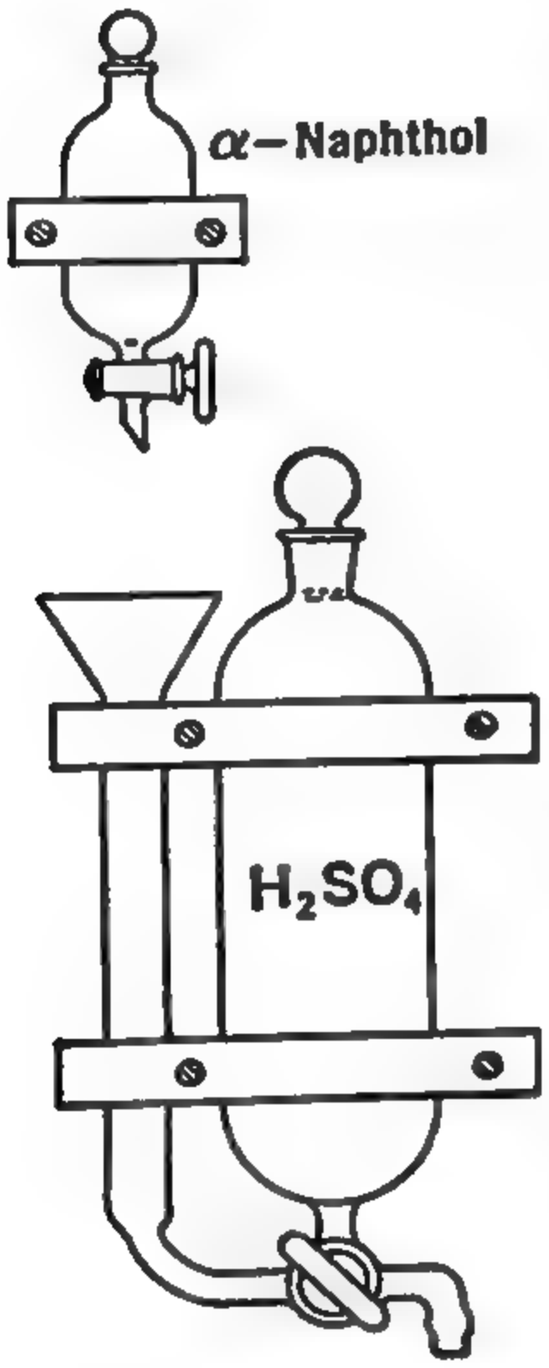
The color reactions with the phenols are performed in various ways. The test with α-naphthol, for example, which is perhaps used more frequently than any of the others, is made as follows: 1 to 2 ml. of the sugar solution is treated in a test tube with 1 to 2 drops of a 10 to 20 per cent alcoholic solution of α-naphthol. A few milliliters of concentrated sulfuric acid (must be free from nitric acid) are then carefully added so as to flow down the walls of the tube to the bottom. If sugars containing a ketone group are present a violet ring will form instantly at the junction of the two liquids; in the presence of aldoses a gentle warming of the test tube is usually necessary in order to bring out the full intensity of color. The α-naphthol test, which is of extreme delicacy, is frequently employed in sugar houses and refineries in testing

[32] *Bull. soc. chim. biol.*, **9**, 928 (1927).

[33] *Ber.*, **64B**, 2856 (1931); **65**, 1110 (1932).

the condensation water from the vacuum pan for presence of sucrose lost by entrainment.

Various apparatus have been developed for making routine tests with α-naphthol; the one designed by Spencer is shown in Fig. 264.[34] The funnel tube is first rinsed with water, then with the sample, and finally filled with the sample to the bend of the funnel tube by closing the three-way stopcock. A few drops of α-naphthol are added from the reservoir on top, and the cock is turned to admit a few milliliters of sulfuric acid. After the color reaction has been observed the funnel tube is drained and rinsed with water, and is then ready for the next test.



(Reproduced with permission from Ind. Eng. Chem., 15, 593.)

FIG. 264. Spencer's apparatus for the α-naphthol test.

If the reaction described for α-naphthol is carried out with thymol, menthol, resorcinol, and other phenols similar colorations are produced, the tints varying from cherry red to deep purple.

Some sugar chemists prefer lysol or cresol to α-naphthol, because the solutions of these phenols keep better on standing, although they are not so sensitive as the naphthol reagent. Lysol is diluted with 5 parts of distilled water, and the test is made as with naphthol. The cresol reagent, according to Stevens,[35] is prepared by dissolving 6 g. castile soap in 100 ml. water, and mixing with 15 ml. cresol. The mixture is warmed and agitated, and more soap is added if necessary. The solution to be tested for sugar is poured into a test tube to the height of about 1 inch, 5 to 10 drops of cresol reagent is added and thoroughly mixed with the solution, and the tube is then cooled in running water. Then concentrated sulfuric acid is run below the aqueous layer to a height of about ½ inch. The tube is gently rolled between the fingers. In the presence of 0.0001 per cent of sugar a light pink ring forms in 1 to 2 hours; with 0.001 per cent a pink ring appears almost immediately, and with increasing concentrations the color becomes red to reddish black. Impurities, like iron or lime salts, which interfere with the α-naphthol test, have no effect.

The tests with phenols and hydrochloric acid are usually made by warming a few milliliters of the sugar solution with a solution of the phenol (resorcinol, orcinol, phloroglucinol, etc.) in concentrated hydrochloric acid. The colorations thus obtained are usually very brilliant,

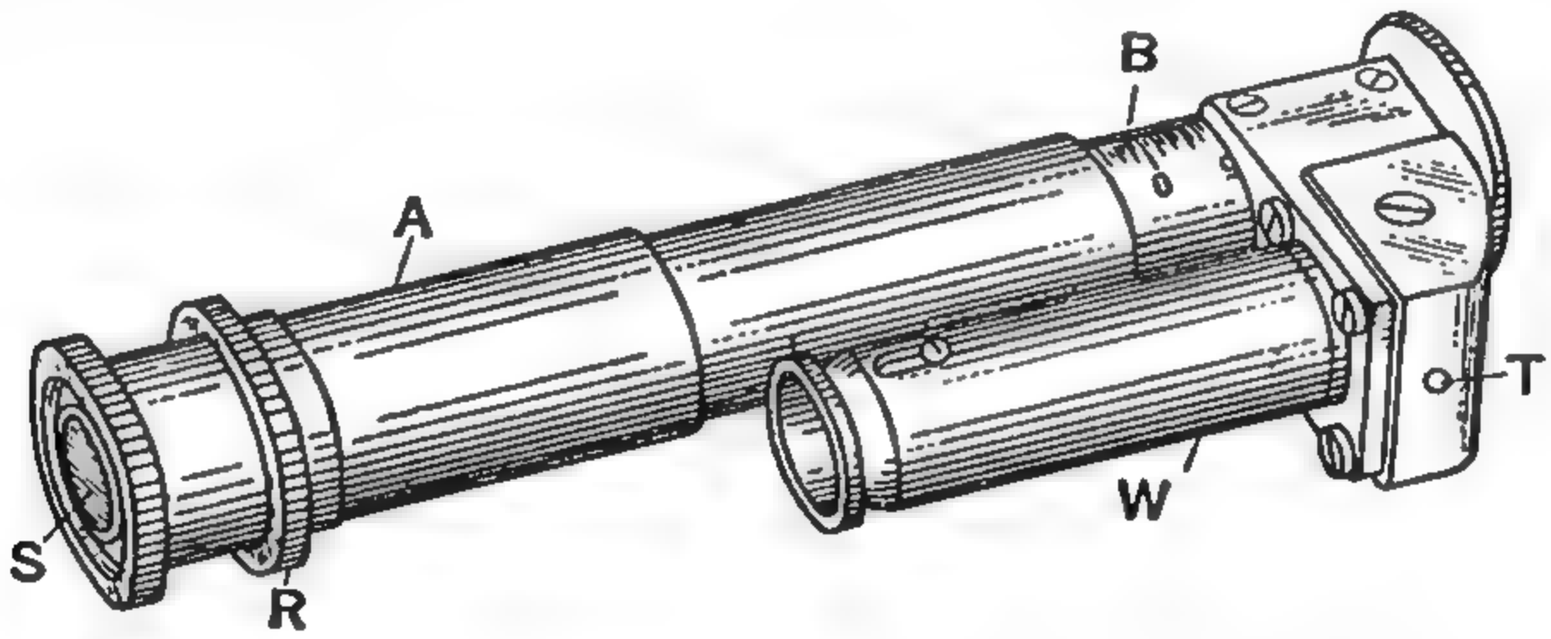
[34] *Ind. Eng. Chem.*, **15**, 593 (1923).
[35] *Ind. Eng. Chem.*, **15**, 363 (1923).

varying in tint from a bright red to a bluish violet. The colors formed are not permanent, however; they rapidly darken, and the clear-colored solution soon becomes turbid with the precipitation of a dark-colored condensation product.

Glacial acetic acid may also be used instead of hydrochloric acid to dissolve the phenols employed in these tests. Wong[36] has found that under these conditions the solutions remain clear and can be used directly for spectroscopic examination or for colorimetric comparisons.

USE OF THE SPECTROSCOPE IN STUDYING COLOR REACTIONS FOR SUGARS

The spectroscope has been used with great success by Tollens and his coworkers in studying the colors obtained by treating sugars with different reagents. The appearance of characteristic absorption bands in different parts of the spectrum, when the colored solution is viewed through the spectroscope against white light, is peculiar to many sugars.



(Courtesy of Carl Zeiss, Inc.)

FIG. 265. Direct-vision spectroscope.

Description of Direct-Vision Spectroscope. A simple type of spectroscope for studying absorption spectra is the direct-vision instrument illustrated in Fig. 265, the interior construction of which is shown in Fig. 266.

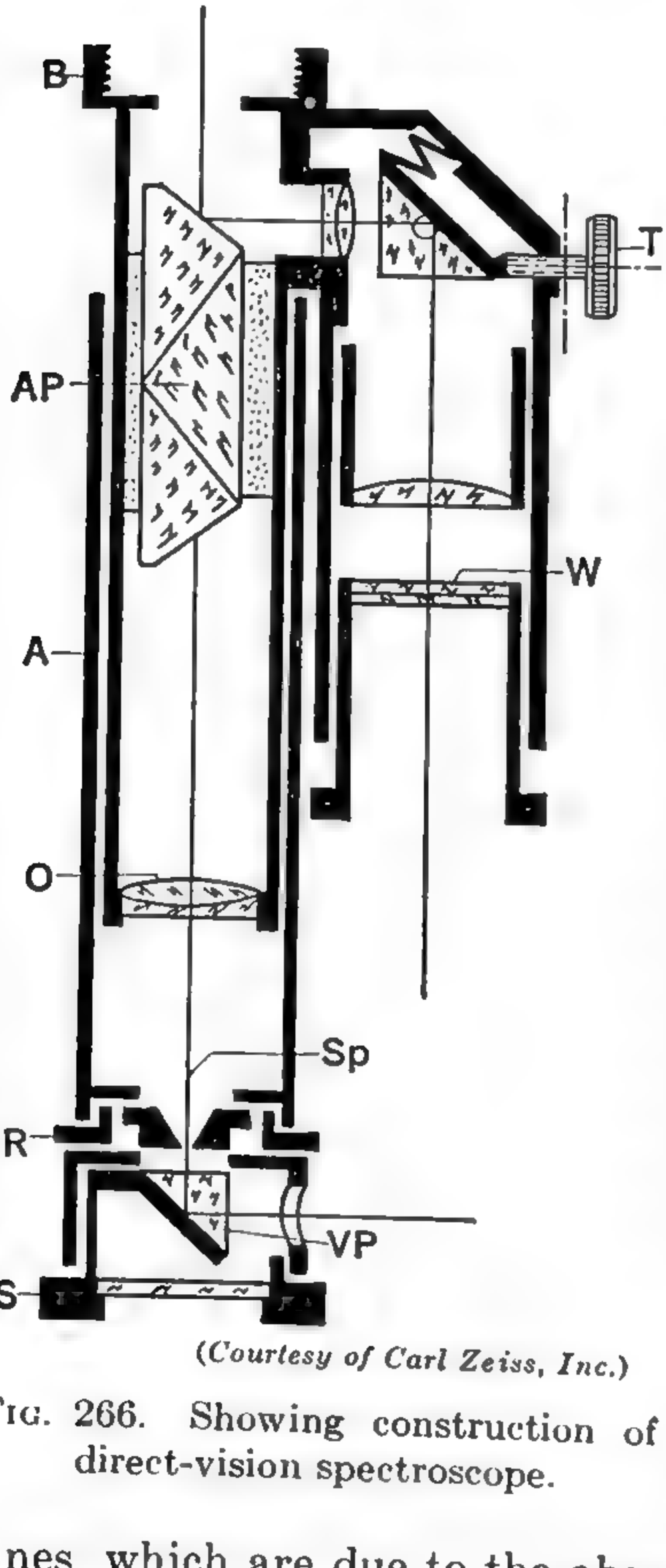
The essential parts of the apparatus consist of a telescopic tube containing an Amici prism *AP* and an achromatic objective *O*. At one end of the tube, protected by the window *S*, a diaphragm is situated containing a slit *Sp*, the width of which can be adjusted by turning the milled ring *R*. One half of the slit is covered with a small comparison prism *VP*, used to obtain two spectra in juxtaposition, as explained below.

At the other end of the spectroscope is attached a small lateral tube containing wavelength scale *W*, a converging lens, and a right-angle prism, from the hypotenuse surface of which the image of the scale *W*

[36] *Chinese J. Physiol.*, 2, 255 (1928).

is reflected through an achromatic objective upon the front surface of the Amici prism. The right-angle prism can be adjusted by means of screw *T*.

If the slit end of the spectroscope is pointed towards a sodium flame the rays of light will pass into the spectroscope in the direction of its axis. Ring *R* is turned to make the slit very narrow, and the telescope is focused by shifting the slit tube with respect to the ocular tube until a sharply defined image of the uncovered half of the slit is obtained by the light passing directly through the slit to the eye. The image of the scale *W* is reflected at the same time onto the surface of the Amici prism from the lateral tube. The small tube holding *W* is adjusted so that there is no parallax between the spectrum and the scale. The position of the sodium line is noted upon the wavelength scale, and if it is not at 589 mμ it is set in the correct position by means of screw *T*. If the spectroscope is now directed toward the sky a continuous spectrum is obtained. The half of the slit which is covered by prism *VP* may be illuminated through a lateral opening by means of a small mirror, and in this way the entire length of the slit furnishes a continuous spectrum.

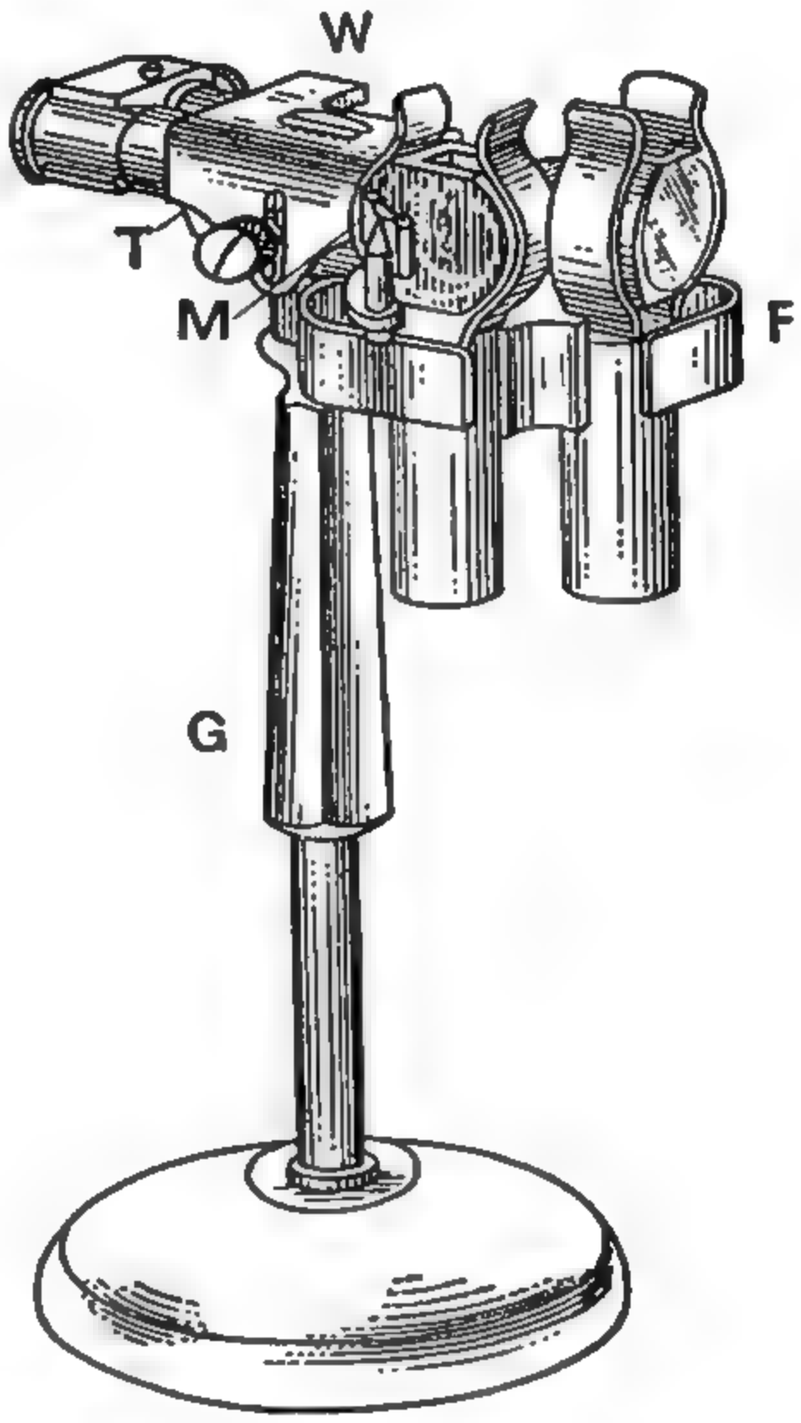


(*Courtesy of Carl Zeiss, Inc.*)

FIG. 266. Showing construction of direct-vision spectroscope.

If the slit has been sufficiently reduced in width, the spectrum of sunlight is seen to be crossed by a number of dark lines, the so-called Fraunhofer lines, which are due to the absorption of certain rays of light from the incandescent mass of the sun by the vaporized elements of the solar atmosphere. A dark line (the D line of Fraunhofer's scale), for example, corresponds to the position of the bright-yellow line obtained with the sodium flame and so of the other elements. The position and wavelength of the more important Fraunhofer lines is shown in Fig. 272; their presence is very helpful in defining the position of absorption spectra.

For studying absorption spectra the spectroscope is mounted upon a stand as shown in Fig. 267. The spectroscope is held in position by a clamp *W*. The solution to be examined is placed in an absorption cell or a test tube, in a special holder which fits into spring clip *F*. It is viewed against white light reflected by mirror *M* meanwhile solution will cause characteristic dark bands to appear upon that part of the spectrum corresponding to the lower half of the slit. The part of the spectrum corresponding to the half of the slit which is covered by prism *VP* and is caused by the beam of white light reflected by mirror *M* meanwhile remains continuous; together with the wavelength scale it serves for the exact location of the absorption bands.



(*Courtesy of Carl Zeiss, Inc.*)

FIG. 267. Direct-vision spectroscope, mounted for study of absorption.

The absorption spectra of two solutions may also be compared by placing another cell or test tube of the same dimensions as the first between the mirror *M* and the prism *VP*, as shown in the figure. The eye of the observer may be shaded from the light source by placing a cardboard screen around the ocular of the spectroscope.

Solutions which are only weakly absorptive are best examined through a long absorption tube which may be placed in a special holder between the light source and the spectroscope.

Tollens's Method of Studying Absorption Spectra. In preparing color tests of sugar solutions for spectroscopic examination it is important that the color remain permanently in solution and that no turbidity develop which would obscure the visible parts of the spectrum. This is sometimes accomplished by carrying out the reaction in the presence of alcohol or some other solvent to hold the color compound in solution. A better way is by use of Tollens's[37] deposit method ("Absatzmethode"). In this method the deposit of insoluble condensation products obtained by treating the sugar solution with hydrochloric acid and the phenol (orcinol, phloroglucinol, naphthoresorcinol, etc.) is filtered off, washed several times with water, and then dissolved

[37] *Ber.*, **29**, 1202 (1896).

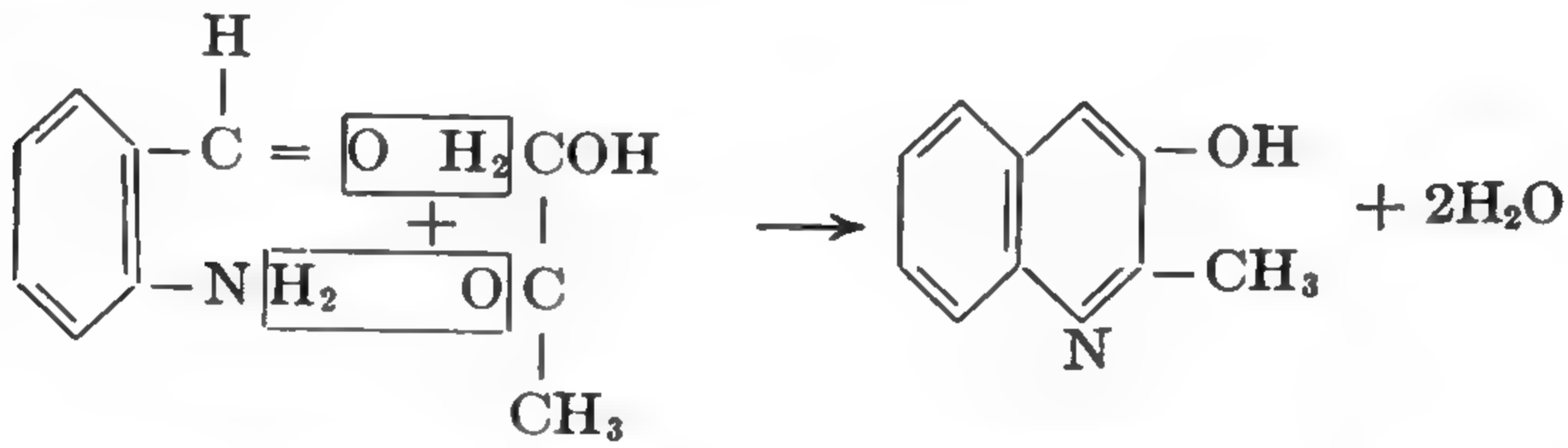
in alcohol. Bright-colored solutions are thus obtained which can be brought by dilution with alcohol to the degree of intensity suitable for spectroscopic examination. Descriptions of characteristic absorption spectra will be given under the reactions for groups and individual sugars.

Of less importance than the color reactions with phenols are the color tests obtained by treating sugars with aromatic amines (aniline, xylidine, diphenylamine, etc.) in the presence of concentrated hydrochloric acid. The colors in this instance are due to a combination between the aromatic amine and the furfural, methylfurfural, and hydroxymethylfurfural derived from the decomposition of the sugar.

If 1 ml. of a sugar solution is heated for 10 minutes in a boiling-water bath with 9 ml. of 77 per cent sulfuric acid and 0.3 ml. of a 1 per cent solution of indole in alcohol, an intensive brown coloring matter is formed.[38]

Urea and guanidine with sulfuric acid also give characteristic color reactions with sugars.[39]

In this connection may also be mentioned the acetol test for sugars, developed by Baudisch and Deuel,[40] who found that all the simple carbohydrates, including the pentoses, upon distillation with sodium bicarbonate yield acetol, $CH_3—CO—CH_2OH$. The acetol is identified by coupling it with *o*-aminobenzaldehyde, which reaction yields 3-oxyquinaldine:



o-aminobenzaldehyde + acetol = 3-oxyquinaldine + water

The oxyquinaldine is readily recognized by its intense bluish fluorescence in sodium bicarbonate solution.

To a solution of 0.1 g. of sugar dissolved in 100 ml. of water, add 5 g. of solid sodium bicarbonate and distil the solution nearly to dryness. To the distillate add 30 mg. of *o*-aminobenzaldehyde and enough potassium hydroxide to make the solution distinctly alkaline. Put in a few pieces of porous plate to prevent bumping, evaporate to one-third the original volume over a free flame, cool, and acidify with hydrochloric

[38] Dische and Popper, *Biochem. Z.*, **175**, 371 (1926).
[39] Foulger, *J. Biol. Chem.*, **99**, 207 (1932).
[40] *J. Am. Chem. Soc.*, **44**, 1885 (1922).

acid. Add solid sodium bicarbonate again until the solution is alkaline to litmus. A strong bluish fluorescence is observed which can be seen in daylight, but more strongly in light of short wavelength as that of the iron arc. To confirm the test, extract the oxyquinaldine by shaking out with alcohol-free ether, dry with a little anhydrous sodium sulfate, and distil off the ether. White crystals of oxyquinaldine remain which are dissolved in alcohol; upon addition of water a strong fluorescence is observed. Five milligrams of reducing sugar gives a positive test, but larger quantities of non-reducing sugars like sucrose are required, because of their resistance to attack by alkali.

III. HYDRAZONE AND OSAZONE REACTIONS OF REDUCING SUGARS WITH PHENYLHYDRAZINE AND ITS SUBSTITUTED DERIVATIVES

In many respects the most important of the qualitative tests for sugars are those obtained with phenylhydrazine and its substituted derivatives. Phenylhydrazine was introduced as a reagent in sugar chemistry by Emil Fischer[41] in 1884; it has been of immense service not only as a means of separation and identification but also in first opening a way to a thorough understanding of the molecular constitution of sugars.

Hydrazone Reaction. The reaction with phenylhydrazine is limited to such sugars as contain a free carbonyl group and proceeds in two phases with production of two entirely different classes of compounds. The first phase of the reaction is common to all aldehydes and ketones, the O of the carbonyl group combining with H_2 of the amino group in the phenylhydrazine with formation of a group of compounds called hydrazones. With formaldehyde, for example, the reaction proceeds as follows:

$$\underset{\text{Formaldehyde}}{H_2C:O} + \underset{\text{Phenylhydrazine}}{H_2N-NHC_6H_5} \longrightarrow \underset{\text{Formaldehyde-phenylhydrazone}}{H_2C:N-NHC_6H_5} + \underset{\text{Water}}{H_2O}$$

With the carbonyl group of a sugar the reaction would be for a diose:

$$\begin{array}{l} CH_2OH \\ | \\ HC:O \\ \text{Diose} \end{array} + \underset{\text{Phenylhydrazine}}{H_2N-NHC_6H_5} \longrightarrow \begin{array}{l} CH_2OH \\ | \\ HC:N-NHC_6H_5 \\ \text{Diose-phenylhydrazone} \end{array} + \underset{\text{Water}}{H_2O}$$

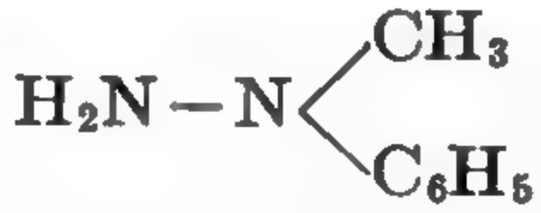
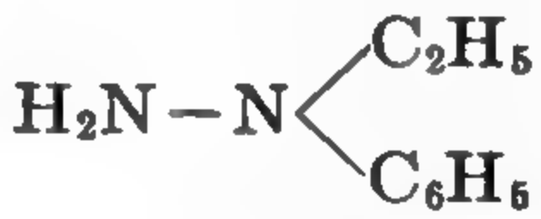
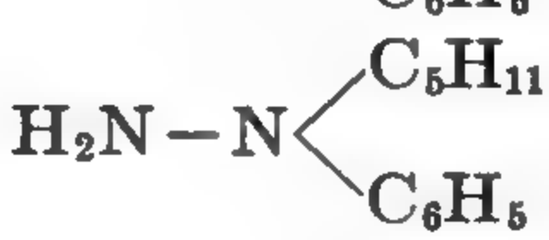
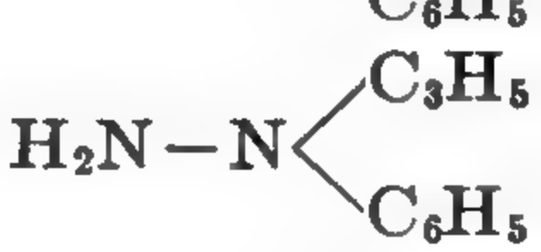
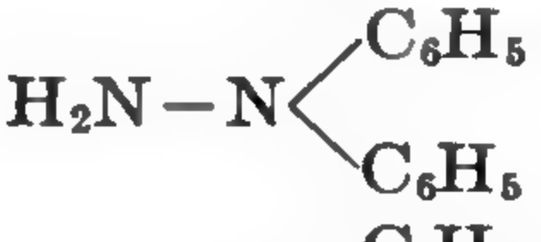
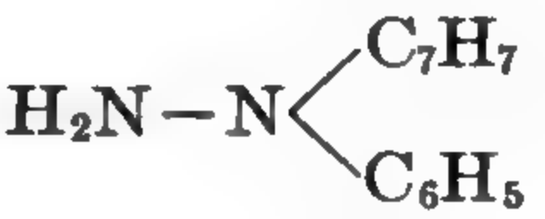
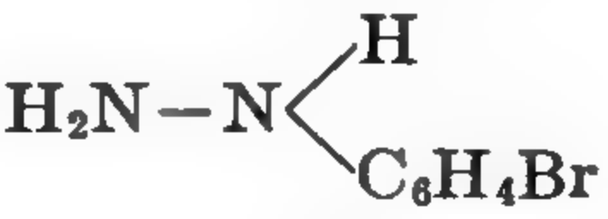
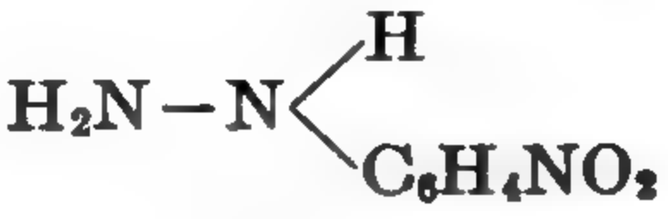
The hydrazone reaction is carried out by treating the sugar solution in the cold with a solution containing 1 volume of phenylhydrazine, 1 volume of 50 per cent acetic acid, and 3 volumes of water. A little more of the phenylhydrazine is used in making the test than the

[41] *Ber.*, 17, 579 (1884).

theoretical quantity corresponding to the supposed amount of sugar present. In place of the above solution the crystalline chloride of phenylhydrazine may be used to advantage, a few grams of sodium acetate also being added to promote the reaction. After the above treatment the hydrazones of the sugars will separate sooner or later as well-defined crystalline compounds, the length of time for separation depending upon the solubility of the hydrazones formed. The phenylhydrazone of mannose, for example, being very insoluble, will separate almost immediately; those of the methylpentoses, fucose, rhamnose, and rhodeose also deposit readily; the phenylhydrazone of glucose, on the other hand, which is quite soluble in water, may require 1 or 2 days for its precipitation. By filtering off the hydrazones as they are formed a separation of sugars in mixtures may often be accomplished.

After separation of the hydrazones the latter are filtered off and recrystallized either from water or, in case of difficultly soluble hydrazones, from alcohol or pyridine.

Use of Substituted Derivatives of Phenylhydrazine. In place of phenylhydrazine any of its substituted derivatives may be used for the purpose of precipitating sugars. The substituted phenylhydrazines yield in many cases characteristic hydrazones with sugars and their use in sugar chemistry has been of the greatest service. Of the various substituted phenylhydrazines the following are among the most important.

1. Methylphenylhydrazine 
2. Ethylphenylhydrazine 
3. Amylphenylhydrazine 
4. Allylphenylhydrazine 
5. Diphenylhydrazine 
6. Benzylphenylhydrazine 
7. Parabromophenylhydrazine 
8. Paranitrophenylhydrazine 

Other hydrazines than those of the phenol group are also employed, as, for example,

9. Naphthylhydrazine $H_2N-N\begin{matrix}H\\ \diagup\\ \diagdown\\ C_{10}H_7\end{matrix}$

The reactions with the substituted hydrazines are usually best carried out in alcoholic solution, the hydrazones formed being for the most part much less soluble than those of ordinary phenylhydrazine.

In the examination of the hydrazones obtained from sugar solutions a melting point of the product is taken before and after recrystallization. If the melting point remains unchanged the hydrazone is pure. Should a difference in the temperature of melting be obtained the hydrazone should be recrystallized until successive determinations show no change in melting point. A table of melting points will then usually identify the hydrazone of the sugar. (See pp. 684–688.)

Separation of Sugars from Hydrazones. When a sufficient quantity of hydrazone is available it is always well to decompose the compound and make a direct examination of the separated sugar. For the separation of sugars from their hydrazones two processes are available: First, by means of concentrated hydrochloric acid as originally used by Fischer. Second, by means of benzaldehyde and formaldehyde as recommended by Herzfeld[42] and by Ruff.[43]

When the hydrazone of a sugar is treated with concentrated hydrochloric acid the chloride of the hydrazine and free sugar are formed:

$$\underset{\text{Hexose phenylhydrazone}}{C_6H_{12}O_5N-NHC_6H_5} + HCl + H_2O \rightarrow \underset{\text{Hexose}}{C_6H_{12}O_6} + \underset{\text{Phenylhydrazine chloride}}{HCl\,H_2N-NHC_6H_5}$$

The phenylhydrazine chloride is almost insoluble in concentrated hydrochloric acid and is removed by filtration. The filtrate is neutralized with lead carbonate; the lead chloride is filtered off, and the filtrate evaporated to a sirup. The sirup is shaken with 95 per cent alcohol, any remaining lead chloride filtered off, and the alcoholic filtrate evaporated to a sirup which is set aside for the sugar to crystallize.

The separation of sugars from their hydrazones by means of aldehydes is much simpler than by use of hydrochloric acid, and this is the process most generally used at present. For this purpose benzaldehyde is usually employed for the hydrazones of phenylhydrazine and formaldehyde for the hydrazones of the substituted hydrazines.

[42] *Ber.*, **28**, 442 (1895).
[43] *Ber.*, 32, 3234 (1899).

The reaction between the aldehyde and hydrazone is a simple one, the aldehyde displacing the sugar with formation of aldehyde hydrazone.

$$\underset{\text{Hexose phenylhydrazone}}{C_6H_{12}O_5N-NHC_6H_5} + \underset{\text{Benzaldehyde}}{C_6H_5CHO} \longrightarrow \underset{\text{Hexose}}{C_6H_{12}O_6} + \underset{\substack{\text{Benzaldehyde}\\ \text{phenylhydrazone}}}{C_6H_5CHN-NHC_6H_5}$$

$$\underset{\text{Hexose diphenylhydrazone}}{C_6H_{12}O_5N-N(C_6H_5)_2} + \underset{\text{Formaldehyde}}{CH_2O} \longrightarrow \underset{\text{Hexose}}{C_6H_{12}O_6} + \underset{\substack{\text{Formaldehyde}\\ \text{diphenylhydrazone}}}{CH_2N-N(C_6H_5)_2}$$

The reaction is best carried out by treating a solution of the hydrazone in 50 per cent alcohol in a flask with an amount of the aldehyde slightly in excess of the theoretical quantity necessary to effect decomposition. The flask is then attached to a reflux condenser and the solution gently boiled for an hour. After cooling, the solution is filtered from the aldehyde hydrazone, the filtrate shaken out several times with ether in a separatory funnel, the sugar solution, after decolorizing with animal charcoal, evaporated to a sirup and set aside for crystallization. Should crystallization not take place immediately, the process may be promoted by priming the sirup with a minute crystal of the sugar suspected to be present. After crystallization the sugar crystals are filtered off, washed with alcohol and ether (using suction), and dried between filter papers in a desiccator over concentrated sulfuric acid. The identity of the sugar thus obtained is then established by determination of its specific rotation.

Instead of the aldehydes already mentioned, *p*-nitrobenzaldehyde has been recommended by Votoček and Valentin,[44] and acetaldehyde by Collatz and Neuberg-Rabinovitch.[45]

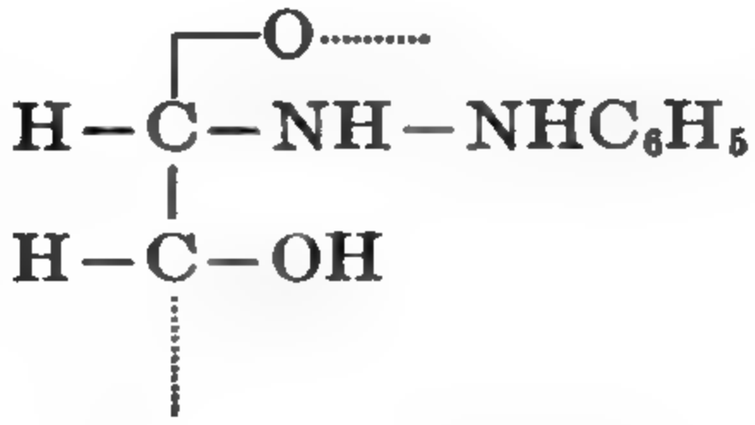
If the filtrate obtained from filtration of a hydrazone is shaken out with ether to remove excess of hydrazine, the solution can be treated a second time with a different hydrazine. In this manner a qualitative separation of several mixed sugars may be accomplished.

Isomeric Hydrazones. A peculiarity of a number of hydrazones is the existence of two or more isomers of different crystalline form, melting point, and specific rotation. Thus the *d*-glucose phenylhydrazone of Fischer and Tafel[46] melts at 144–146°, has an initial specific rotation of —66.57, and a final rotation of —52.00. Behrend and Lohr[47] obtained two phenylhydrazones of *d*-glucose, one melting at

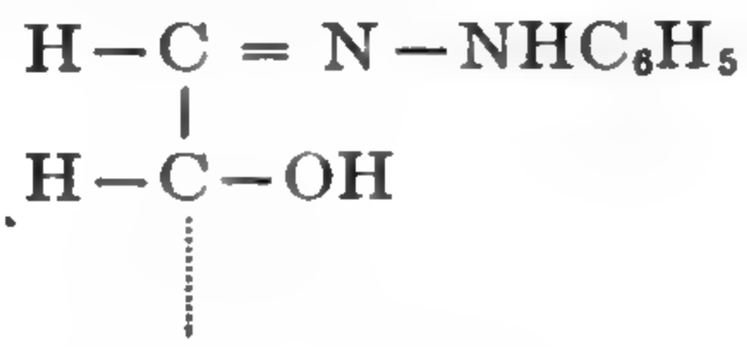
[44] *Arhiv Hem. Farm.*, **5**, 155 (1931).
[45] *Biochem. Z.*, **255**, 27 (1932).
[46] *Ber.*, **20**, 2566 (1887).
[47] *Ann.*, **353**, 106 (1907); **362**, 78 (1908); **377**, 189 (1910).

159–160° and with an initial rotation of —87, the other melting at 140–142° and with an initial rotation of —2. Both gave an equilibrium rotation of —50.[48]

Theoretically, the hydrazones may exist either as ring compounds (hydrazides) with the characteristic group



or as chain compounds (Schiff bases) with the characteristic group



The ring forms will exhibit mutarotation, due to α–β isomerism, while the chain compounds may exist in the syn- or anti-form, with different melting points. In the case of some sugars the configuration of the hydrazones has been definitely established; in others it is unknown, and it is quite probable that some hydrazones consist of mixtures of several modifications.

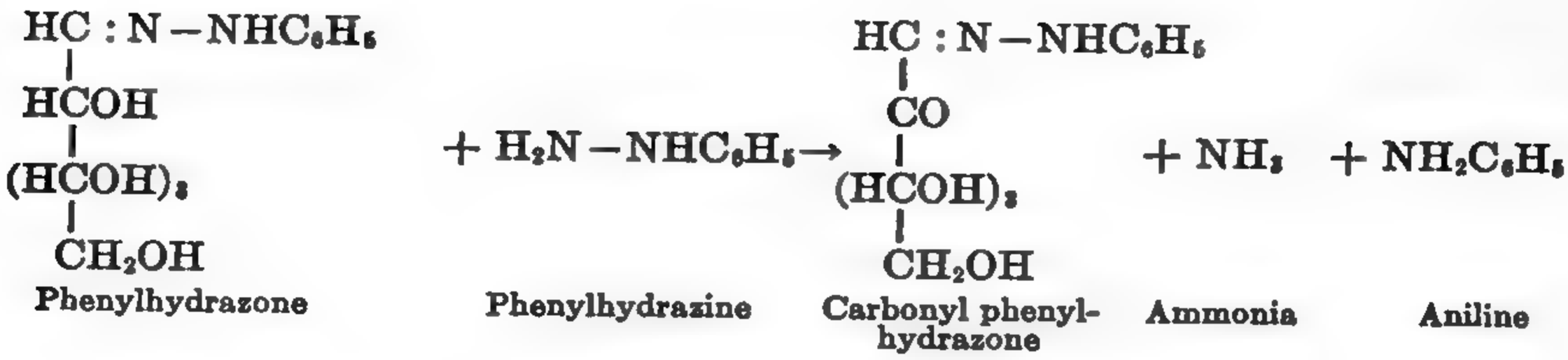
Osazone Reaction. While the hydrazone reaction is of preeminent value in the isolation of sugars, the osazone test with phenylhydrazine is usually of more qualitative significance owing to the greater insolubility of the osazones in water and the consequent greater rapidity and ease of their separation as compared with hydrazones.

If a solution of a reducing sugar is treated with a large excess of phenylhydrazine and then warmed, two molecules of phenylhydrazine unite with the sugar molecule, forming an osazone. The aldehyde or ketone group of the sugar and the *adjacent* alcohol group are the ones which always participate in this reaction.

One molecule of phenylhydrazine reacts with the hexose to form the phenylhydrazone as shown on p. 665. A second molecule of phenylhydrazine acts as an oxidizing agent, and is itself reduced to aniline and

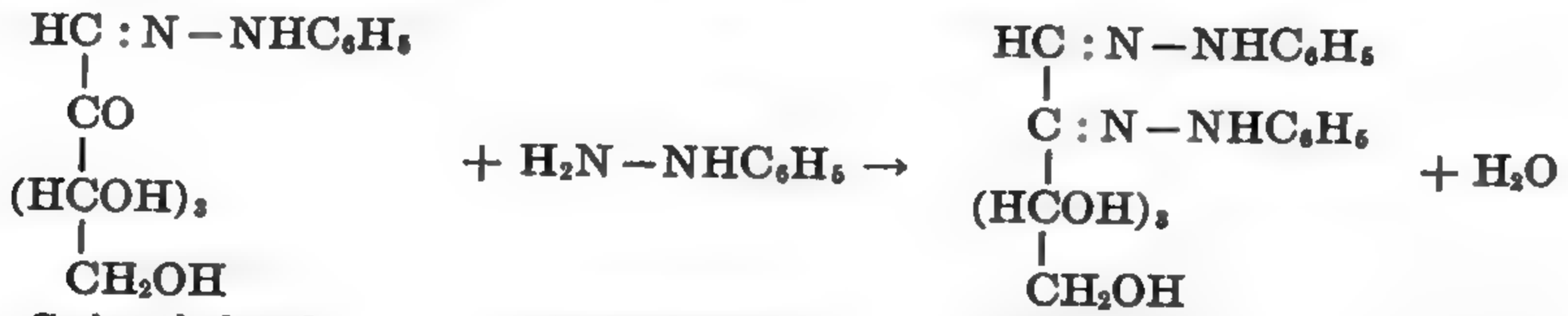
[48] For further examples of mutarotating hydrazones see the list on pp. 684–688, under *l*-rhamnose and *d*-mannose.

ammonia:



Phenylhydrazone | Phenylhydrazine | Carbonyl phenylhydrazone | Ammonia | Aniline

A third molecule of phenylhydrazine reacts simultaneously with the newly formed carbonyl group, and the osazone is formed:



Carbonyl phenylhydrazone | Phenylhydrazine | Phenylosazone | Water

In conducting the reaction for osazones the original method of Fischer[49] is usually followed. For 1 g. of sugar, 2 g. of phenylhydrazine chloride and 3 g. crystallized sodium acetate ($CH_3COONa + 3\,H_2O$) and 20 ml. of water are heated together for ¾ to 1½ hours in a large test tube of about 50-ml. capacity placed in a boiling-water bath. The contents of the tube are stirred occasionally to promote crystallization. Instead of the chloride one may employ a solution of phenylhydrazine acetate, prepared by adding concentrated acetic acid drop by drop to phenylhydrazine until the turbid emulsion clears. The osazone reaction with the substituted hydrazines is conducted in the same way as with phenylhydrazine.

The osazones of the sugars are yellowish-colored crystalline compounds of variable solubility. The osazones of the monosaccharides crystallize out from the hot solutions; those of the disaccharides, maltose and lactose, however, separate only after cooling. A separation of the osazones of the mono- and disaccharides can be accomplished in this manner, a second crystallization usually rendering the separation complete. While the osazones of the monosaccharides are nearly all of much lower solubility than the corresponding hydrazones, the osazone separation is never complete.

Yield and Time for Formation of Osazones. Sugars differ greatly in the amount of osazone which is formed under a definite method of treatment, and this property has been utilized as a means of identifica-

[49] *Ber.*, 17, 579 (1884).

tion. Maquenne,[50] for example, has determined the yield of osazones obtained by heating 1 g. of different sugars in 100 ml. of water with 5 ml. of a solution, containing 40 g. phenylhydrazine and 40 g. glacial acetic acid in 100 ml., for 1 hour in a boiling-water bath. The sugars studied by Maquenne are arranged in Table XC in the order of yield of osazone.

TABLE XC

YIELD OF OSAZONES AND TIME OF PRECIPITATION FOR DIFFERENT SUGARS

Sugar	Phenylosazone from 1 g. Sugar	Time for Precipitation
	Gram.	
Sorbose	0.82	Turbid in 12 min.
Fructose	0.70	Precipitate in 5 min.
Xylose	0.40	Precipitate in 13 min.
Glucose	0.32	Precipitate in 8 min.
Arabinose	0.27	Turbid in 30 min.
Galactose	0.23	Precipitate in 30 min.
Rhamnose	0.15	Precipitate in 25 min.
Lactose	0.11	Precipitate only on cooling.
Maltose	0.11	Precipitate only on cooling.

It is noted that the ketoses sorbose and fructose are characterized by a much greater yield of osazone. The theoretical yield of osazone from 1 g. of sugar is 2.19 g. for pentoses, 1.99 g. for hexoses, and 1.53 g. for disaccharides. This shows how large a part of even the more insoluble osazones were unprecipitated in Maquenne's experiments. The latter, however, were not intended to give the conditions of maximum yield and were designed simply for purposes of comparison.

Fischer by heating 1 part glucose with 2 parts phenylhydrazine chloride, 3 parts sodium acetate, and 20 parts of water for 1½ hours upon the water bath obtained 85 to 90 per cent of the weight of sugar as osazone. This is nearly three times the amount obtained by Maquenne, but is still less than 50 per cent of the theoretical yield.

Knecht and Hibbert[51] have shown that the osazone reaction becomes quantitative if 12 molecules of phenylhydrazine are added to 1 of sugar (see p. 966).

Mulliken[52] has based a scheme for the identification of pure sugars upon the time of separation of the osazones. Fischer's method of making the test is followed, 0.1 g. sugar, 0.2 g. pure phenylhydrazine chlo-

[50] Maquenne's "Les Sucres," p. 266; *Compt. rend.*, 112, 799 (1891).
[51] *J. Chem. Soc.*, 125, 2009 (1924).
[52] Mulliken's "Identification of Pure Organic Compounds."

ride, 0.3 g. sodium acetate, and 2 ml. water being mixed in a small test tube, corked loosely to prevent evaporation, and heated in boiling water. The tube is shaken occasionally without removing from the bath, and the time for the separation of a precipitate is noted. Under the above conditions Mulliken noted the following:

Sugar	Time for Osazone Separation	Sugar	Time for Osazone Separation
	minutes		minutes
Fructose	2	Arabinose	10
Sorbose	3½	Galactose	15–19
Glucose	4–5	Sucrose	30 (due to slight inversion)
Xylose	7	Maltose	No precipitate in hot solution
Rhamnose	9	Lactose	No precipitate in hot solution

The relation of the sugars as regards time of osazone formation agrees closely with that noted by Maquenne.

Sherman and Williams[53] give the following time of osazone formation for different quantities of sugar under the conditions followed by Mulliken, but with double the quantity of reagents and water.

Time for Precipitation of Osazones

Weight of Sugar Taken	Glucose	Fructose	Invert Sugar	Sucrose
gram	minutes	minutes	minutes	minutes
0.2	4–5	1¼–1½	1½–1⅔	31
0.1	5	1¾–2	2	35
0.05	6½	2½	3	78
0.01	17	5½	6–6½	No ppt.
0.005	34	10	14	
0.0025	65	17		

Sherman and Williams found that with mixtures of different sugars the time of osazone formation was greatly modified. The following results were noted.

Influence of Maltose on Glucose

Weight of Glucose	Weight of Maltose				In Absence of Maltose
	0.2 g.	0.1 g.	0.05 g.	0.01 g.	
gram	minutes	minutes	minutes	minutes	minutes
0.01	No ppt.	40	30	22	17
0.02	26–28				12–13

[53] *J. Am. Chem. Soc.*, **28**, 629 (1906).

INFLUENCE OF LACTOSE ON GLUCOSE

Weight of Glucose	Weight of Lactose				In Absence of Lactose
	0.2 g.	0.1 g.	0.05 g.	0.01 g.	
gram 0.01 0.02	minutes No ppt. 45–48	minutes 50	minutes 32	minutes 25	minutes 17 12–13

INFLUENCE OF SUCROSE ON GLUCOSE

Weight of Glucose	Weight of Sucrose				In Absence of Sucrose
	0.2 g.	0.1 g.	0.05 g.	0.01 g.	
gram 0.005 0.01 0.02	minutes 15–17 14–16 9	minutes 15–17 16	minutes 22 17	minutes 30 17	minutes 33–39 17 12–13

INFLUENCE OF RAFFINOSE ON GLUCOSE

Weight of Glucose	Weight of Raffinose				In Absence of Raffinose
	0.2 g.	0.1 g.	0.05 g.	0.01 g.	
gram 0.005	minutes 27–30	minutes 33–37	minutes 36–38	minutes 37–39	minutes 33–39

INFLUENCE OF MALTOSE ON FRUCTOSE

Weight of Fructose	Weight of Maltose				In Absence of Maltose
	0.2 g.	0.1 g.	0.05 g.	0.01 g.	
gram 0.01	minutes 7–8	minutes 5½–6	minutes 5½–5¾	minutes 5½	minutes 5½

INFLUENCE OF LACTOSE ON FRUCTOSE

Weight of Fructose	Weight of Lactose				In Absence of Lactose
	0.2 g.	0.1 g.	0.05 g.	0.01 g.	
gram 0.01	minutes 9½–10	minutes 7¾	minutes 6¾	minutes 6	minutes 5½

INFLUENCE OF SUCROSE ON FRUCTOSE

Weight of Fructose	Weight of Sucrose				In Absence of Sucrose
	0.2 g.	0.1 g.	0.05 g.	0.01 g.	
gram 0.005	minutes 8⅓	minutes 8¾	minutes 9⅓	minutes 9¼	minutes 9½

The results show that sucrose accelerates, while maltose and lactose retard, the separation of osazone from solutions containing glucose and fructose.

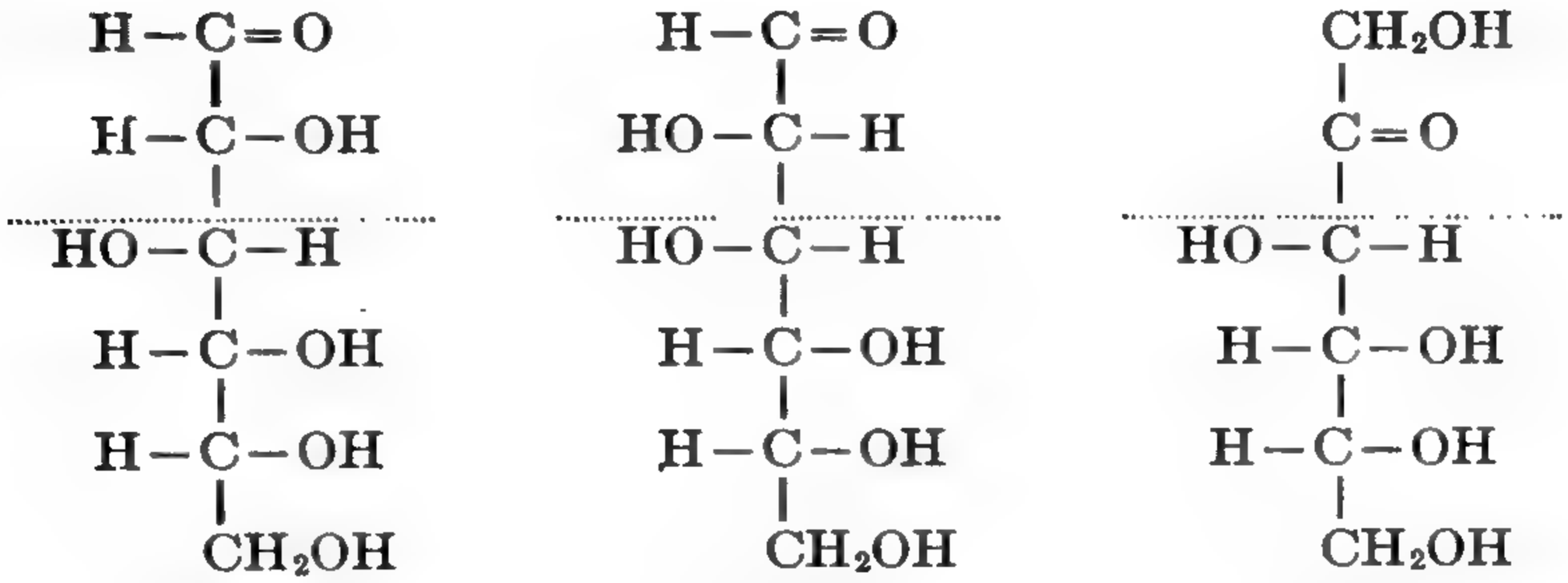
A scheme of identification, based upon yield, or time of formation of osazone under a prescribed method of treatment, is of value only in working with a known quantity of pure sugar. In products containing foreign organic and mineral matter, or a mixture of several sugars, the presence of impurities or of other osazones influences crystallization to a very marked degree. This fact prevents the employment of the osazone reaction for exact quantitative purposes.

The osazones of sugars after precipitation require purification. The crystalline precipitate is filtered off, well washed with cold water, and then pressed as dry as possible between filter papers. The product is then recrystallized from boiling 50 per cent alcohol to which a few drops of pyridine may be added, in case of very insoluble osazones, to promote solubility. Recrystallization may also be effected from acetone and other organic solvents and in case of easily soluble osazones, as of maltose and lactose, from hot water. After dissolving the osazones, the hot solution is filtered and set aside in the cold until crystallization is complete. The purified osazone is then filtered off and dried at a gentle heat. A melting point is then taken which, if the osazone is pure, will remain unchanged after further crystallization. Frequently a table of melting points is sufficient to identify the osazone. (See pp. 684–688.)

Limitations of the Osazone Reaction. The osazone reaction with phenylhydrazine, though invaluable, is not always an absolute test of the identity of a sugar, owing to the fact that a number of isomeric sugars give the same osazone. The pentose sugars *d*-lyxose and *d*-xylose, for example, yield the same phenylosazone of melting point 160–161° C. Similarly the hexose sugars, *d*-glucose, *d*-mannose, and *d*-fructose, yield the same phenylosazone of melting point 206° C. In fact, any of the isomeric sugars which are mutually transformable (as in contact with alkalies) give the same osazone. This is made clearer from the stereoformulas of glucose, mannose, and fructose, shown on p. 675.

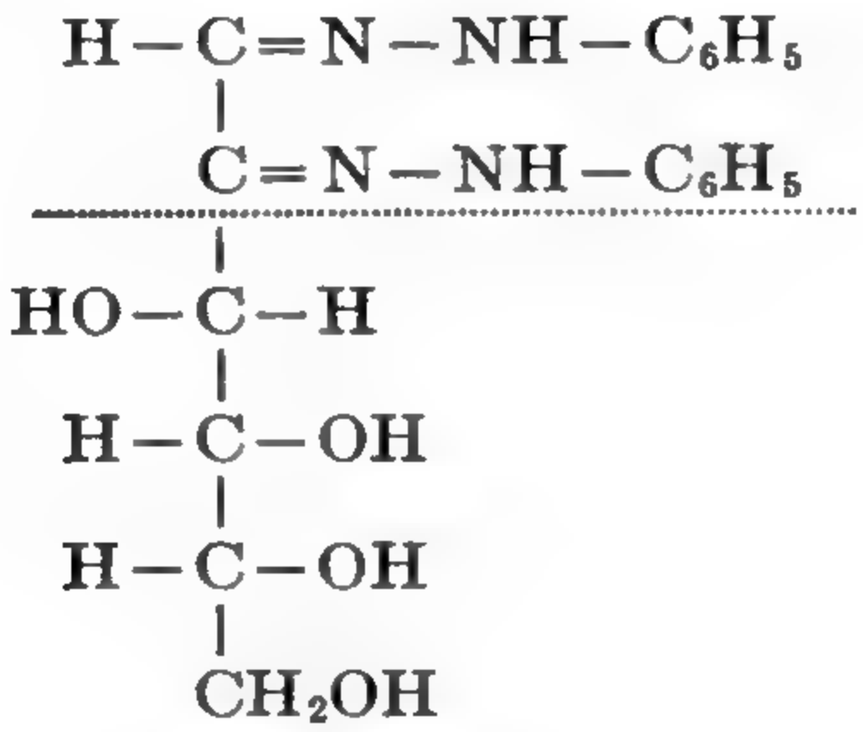
Other examples of sugars giving the same osazone are *d*-arabinose and *d*-ribose; *d*-galactose, *d*-talose, and *d*-tagatose; *d*-allose, *d*-altrose, and *d*-pseudofructose; *d*-idose, *d*-gulose, and *d*-sorbose.

Similarly, the corresponding groups of sugars belonging to the *l*-series, having the same configuration beyond carbon 2, also yield one and the same osazone, differing from the *d*-osazone merely in the direction of the rotation.



d-Glucose *d*-Mannose *d*-Fructose

The part of the molecule below the dotted line has the same spatial arrangement in all three sugars. The part of the molecule above the dotted line is the only part of the molecule affected in the osazone reaction, this in all three sugars giving rise to an osazone which has the same structural formula:



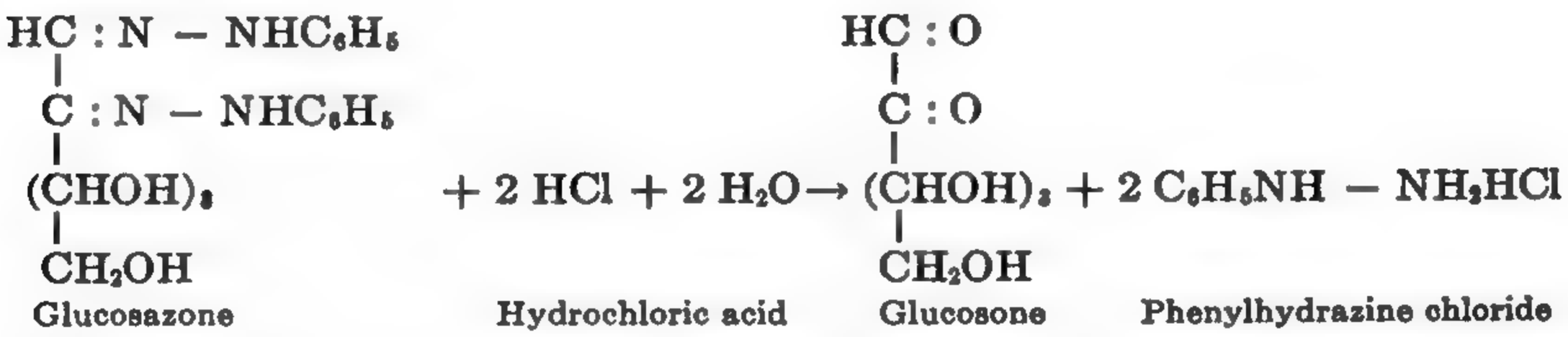
This circumstance, although nullifying the use of phenylosazones in certain cases as a means of identification, has yet thrown a flood of light upon the molecular constitution of sugars.

Test for Ketoses with Methylphenylhydrazine. In distinction from phenylhydrazine the substituted hydrazines do not always give the same osazone reaction with sugars which are mutually transformed. The osazone reaction with substituted hydrazines has, therefore, a distinct qualitative value. Methylphenylhydrazine, for example, forms very readily a characteristic osazone with *d*-fructose, but not with *d*-glucose or *d*-mannose or any of the other aldose sugars. The osazone reaction with methylphenylhydrazine is, therefore, serviceable in distinguishing aldoses from ketoses.

Decomposition of Osazones into Osones. While hydrazones, upon decomposition with strong hydrochloric acid or with benzaldehyde or formaldehyde, yield the component sugar, the osazones cannot be resolved in this manner. The osazone reaction is consequently of value

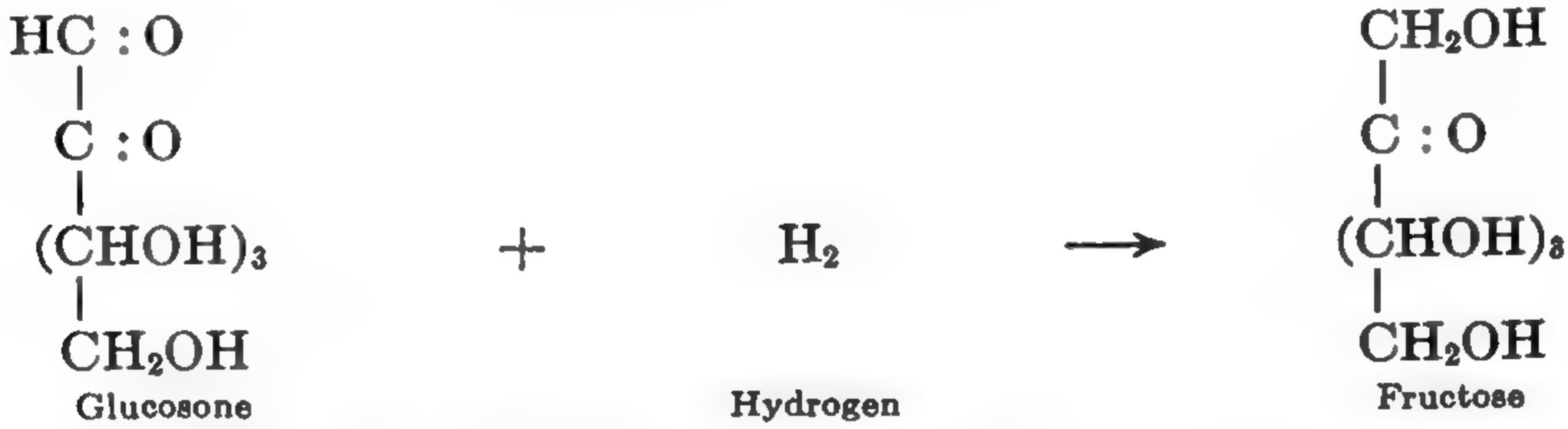
only as a means of identifying and not of separating sugars. The decomposition of osazones with acids and aldehydes has, however, a considerable theoretical interest which may be considered briefly in this connection.

Treatment of osazones with concentrated hydrochloric acid or with certain aldehydes causes, as in the hydrazones, a separation of the phenylhydrazine; the product remaining behind, however, is not the original sugar, but a compound with two adjacent carbonyl groups called an osone. The reaction of glucosazone with hydrochloric acid, for example, is:



The conversion of osazones soluble in hot water into osones can be easily effected with benzaldehyde in the presence of sufficient alcohol, the phenylhydrazine being separated as benzaldehydephenylhydrazone and the osone remaining behind in solution.

Osones upon treatment with zinc dust and acetic acid are reduced by the nascent hydrogen to a sugar, the end carbonyl group being converted always to an alcohol group, as shown in the following equation for glucosone.



It will be seen from the above reaction that the sugar obtained by reduction of an osone is always a ketose. By this means glucose and mannose can be transformed into fructose, and this type of reaction is true for the conversion of any aldose into the corresponding ketose, the steps of the transformation being always

Aldose ⟶ Osazone ⟶ Osone ⟶ Ketose

The osones, though of great service in establishing the relationship of different sugars to one another, have no value either in qualitative or quantitative sugar analysis.

THE IDENTIFICATION OF HYDRAZONES AND OSAZONES

The identification of hydrazones and osazones, by examination of their physical properties, although belonging strictly to the tests for individual sugars, is introduced for convenience at this point.

Determination of Melting Point of Hydrazones and Osazones. The determination of melting point is the principal physical method for identification of hydrazones and osazones.

Capillary-Tube Method. The capillary-tube method is the one most generally employed for determining melting points. The essential requirements for apparatus are shown in Fig. 268. A long-neck flask with a small body of about 20-ml. capacity is filled about two-thirds with pure concentrated sulfuric acid; to prevent discoloration of the acid through accidental contamination with organic matter a small crystal of potassium nitrate, the size of a pinhead, is dropped in. The flask is clamped to a lamp-stand in the manner shown. The opening of the flask is fitted with a perforated cork containing a groove upon the side to allow escape of expanding air. The perforation in the cork should be of such a size as to hold a thermometer, graduated to 300° C., tightly in position; the bulb of the thermometer should be above the bottom of the flask and yet be submerged entirely in the acid.

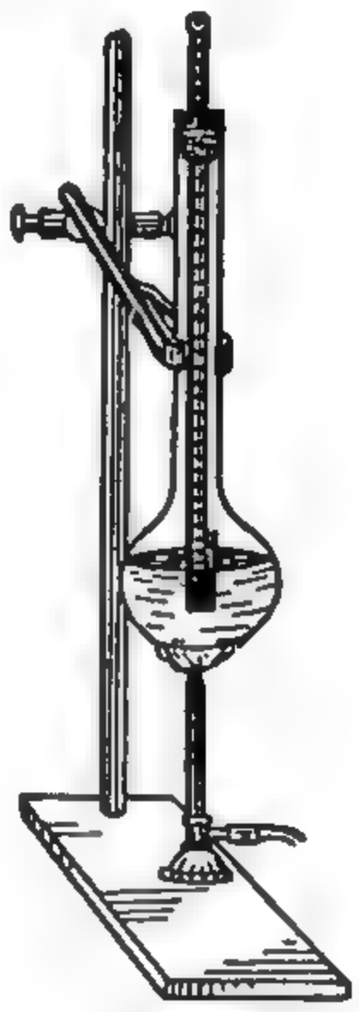


FIG. 268. Apparatus for determining melting points.

This melting-point apparatus has the disadvantage that the acid around the bulb of the thermometer is likely to be heated unevenly unless the flask is very small. In modern practice the sulfuric acid is kept in motion either by means of a stirrer, or by means of convection currents, as in the Thiele-Dennis[54] melting-point tube. But since the early melting-point determinations on hydrazones and osazones were made with the apparatus here described, and as the heating must be done quickly, it is best to follow the older procedure.

Van der Haar[55] recommends paraffin oil in place of sulfuric acid as the bath liquid because it does less damage in case of breakage and because it has a greater coefficient of expansion, so that the correction to the melting point found is smaller. It is best to use sufficient paraffin oil so that its meniscus is slightly above that of the mercury column; in this way the corrected melting point can be read off directly.

[54] *Ind. Eng. Chem.*, 12, 366 (1920).

[55] "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydsäuren," p. 30, 1920. This monograph also discusses exhaustively the identification of sugars by means of the hydrazones, hydrazides, osazones, etc.

The capillary tubes for holding the hydrazone or osazone are best prepared by thoroughly softening a piece of glass tubing by turning it in the flame and then drawing it out to about 1- to 1.5-mm. diameter. By continuing this process backwards along the tube a number of sections are obtained similar to Fig. 269*a*; the sections are then filed off at the points indicated and the smaller ends melted together in the flame. Small tubes of the size and shape shown in Fig. 269*b* are thus obtained.

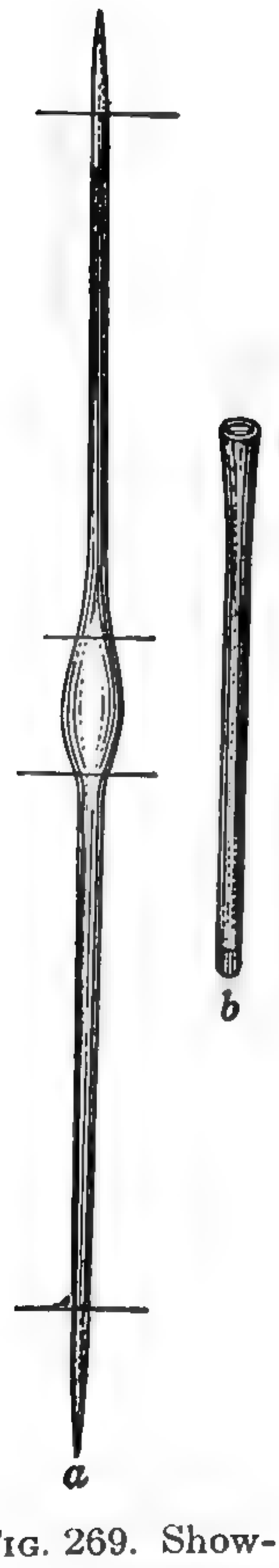


FIG. 269. Showing preparation of capillary tubes for determining melting points.

A small amount of finely powdered hydrazone or osazone is then introduced into the open end of the tube and the tube is gently tapped until the substance has settled to the bottom. To prevent the powdered material from forming too loose a layer it is usually well to push it down tightly by means of a platinum-wire or thin-glass rod. The depth of substance in the tube should not exceed 2 mm. The capillary tube containing the substance is then attached to the thermometer either by binding it with a piece of fine platinum wire or by dipping it first in concentrated sulfuric acid and allowing it to stick to the thermometer bulb by adhesion. The tube is placed so that the layer of substance is even with the center of the mercury bulb.

After the thermometer and tube have been placed in position, as shown in Fig. 268, a small flame is placed beneath the flask and the temperature raised until the liquefaction of the powdered crystals indicates the temperature of melting. Hydrazones and osazones at the point of melting decompose with darkening of color, the evolution of gas causing the liquefied substance to foam upwards in the stem of the tube. The first determination of melting point is only preliminary, and a second and third trial should always be made with fresh tubes and material. The acid in the subsequent tests is heated rapidly to about 5° C. below the melting point first observed and then the temperature raised gradually so that the thread of mercury in the thermometer comes to rest just at the point of liquefaction. The entire operation for glucosazone, for example, melting at 204° to 205° C., should not consume over 4 minutes. Undue protraction of the time of heating affects the result of the determination very markedly, the wide discrepancies noted in the literature between melting-point determina-

tions of the same osazone by different authorities being due largely to this cause.

Maquenne's Block. A second method for determining melting points of hydrazones and osazones is employed considerably by French chemists. This method involves the use of the Maquenne Block, an apparatus invented by Maquenne in 1887, the essential features of which are shown in Fig. 270.

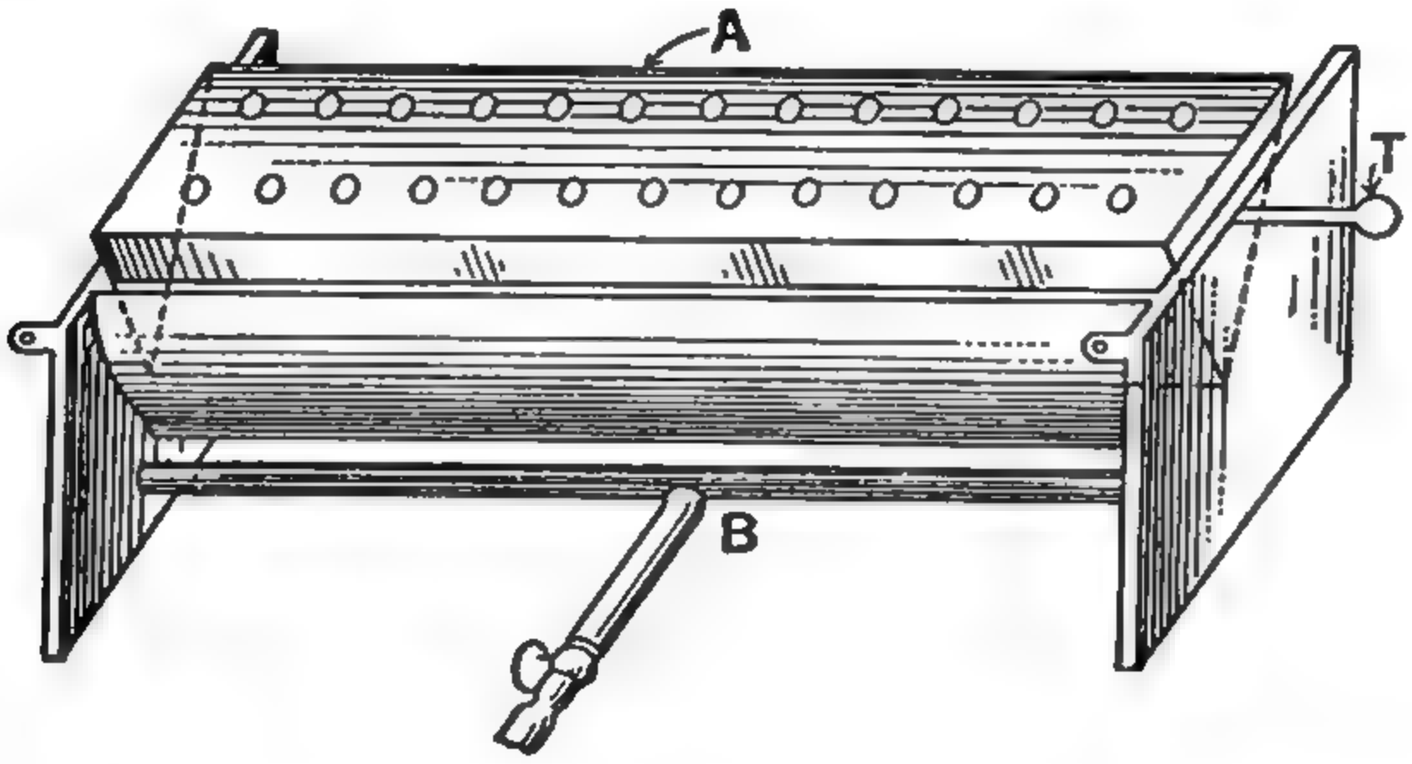


FIG. 270. Maquenne's block for determining melting points.

The important part of Maquenne's apparatus consists of a prismatic block (*A*) of brass, weighing about 2 kilos, which is placed in a frame with one of its edges resting above the openings of a long gas burner (*B*). In one end of the block about 5 mm. below the upper surface a hole is bored, extending nearly the length of the block, into which a thermometer (*T*) can be inserted. In the upper level surface of the block are a number of small, round cavities. In conducting a determination a small amount of substance is placed in one of the cavities, which, to prevent disturbances from air drafts, is covered with a small glass; the thermometer is then inserted so that its bulb is about underneath the cavity, and the burner is started with a low, uniform flame. The temperature is slowly elevated until the substance begins to melt, when the thermometer is drawn out or pushed in until just the end of the mercury thread projects and the temperature is noted. The block is now cooled slightly and a second determination made more slowly than before, using a cavity above the bulb of the thermometer in its second position. Owing to the fact that the block has nearly the same temperature, the entire column of mercury is brought to the same temperature as that of the melting substance and no correction due to contraction of the thread outside the unheated portion of the thermometer is necessary as by the method of melting-point determination previously described.

A comparison of melting points of glucose phenylosazone by the two methods shows the following: capillary tube 205° C. (Fischer), Maquenne Block 230° to 232° C. (Bertrand). From this it would appear that the Maquenne Block gives considerably higher melting points than the capillary-tube method. A critical comparison of the two methods by Müther[56] (see Table XCI) shows, however, that this is not always the case.

It will be seen that Müther obtained for glucosazone results by the block agreeing very closely with those by the tube, the range found by the block being 200° to 206° C. and by the tube 203.5° to 205° C.

TABLE XCI

MELTING POINTS OF HYDRAZONES AND OSAZONES BY DIFFERENT METHODS (Müther)

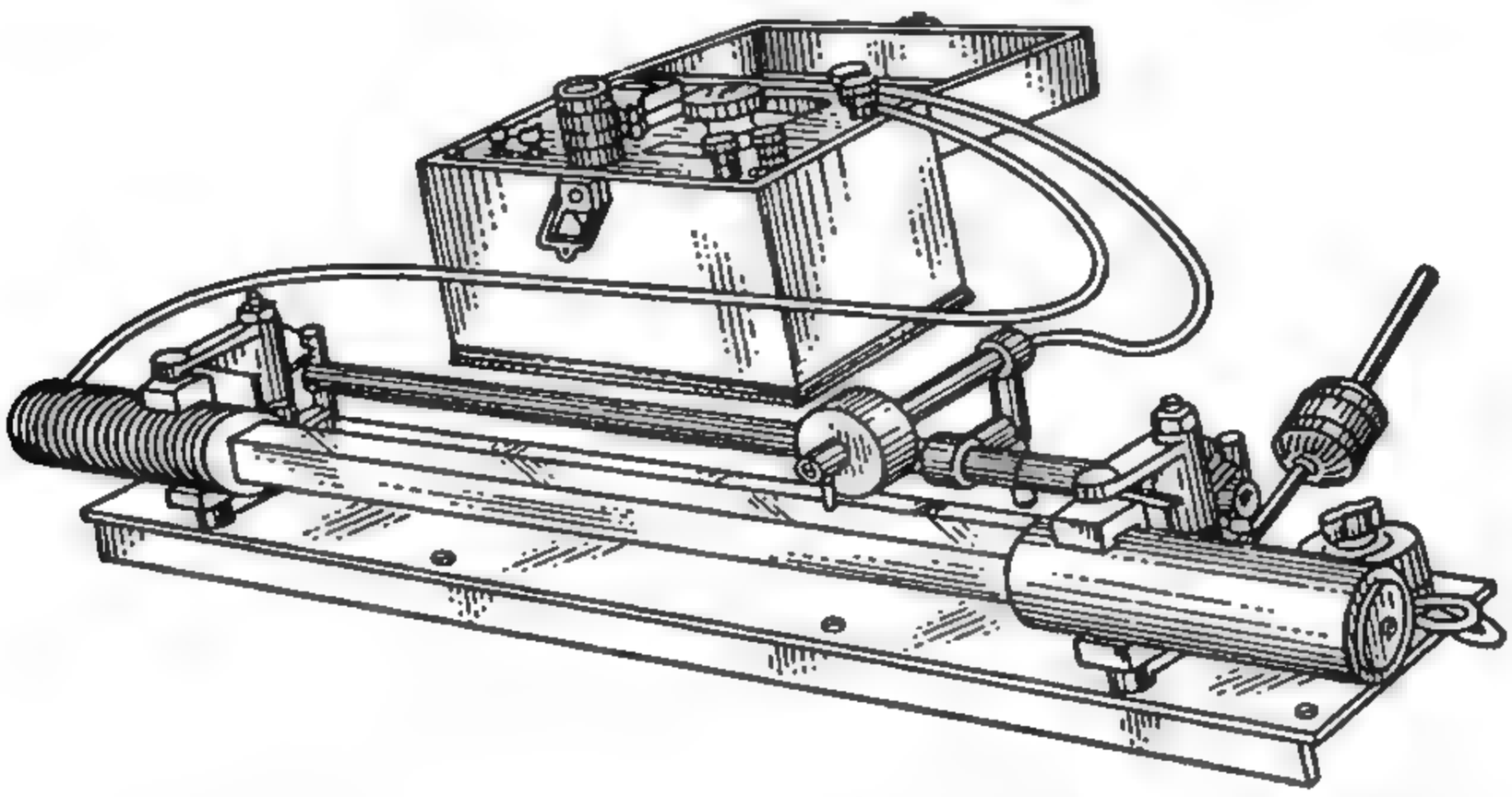
Compound	Method of Melting Point	
	Capillary Tube	Maquenne Block
	°C.	°C.
Arabinose methylphenylhydrazone	164	158–160
		159–160
		159–160
		162
Arabinose diphenylhydrazone	203–204	198
		199–200
Fucose methylphenylhydrazone	177	174–175
		172–173
		170–171
Fucose benzylphenylhydrazone	172–173	165–167
		173–174
Mannose phenylhydrazone	188–189	187
	188–189	191–192
Fructose osazone (glucosazone)	203.5	202–203
	204–205	200–201
	203.5	204–205
	203–204	205–206
	203.5	205

The greater variation by the block is attributed by Müther to the unequal distribution of heat through the brass, the outer surface being more quickly warmed than the center; differences from 3° to 6° C. were also noted for different positions of the thermometer inside the block. The slowness with which the block is heated and cooled and the difficulty with which the cavities are cleaned are also serious objections. With substances which sublime, the Maquenne Block cannot be used on account of the rapid condensation of material from the cavity upon the cover glass. These objections together with the high

[56] Dissertation, Göttingen, 1903.

cost of the apparatus render it much less desirable for determining melting points than the simpler capillary-tube method.

Dennis Melting-Point Apparatus. An apparatus which is based on a similar principle as the Maquenne Block but has none of its disadvantages is the Dennis melting-point apparatus, shown in Fig. 271. It consists of a stout copper bar, with an electrical resistance coil at one end, by which the bar is heated to as high as 300° C. The other end is connected by a copper wire with a potentiometer, reading directly in degrees Centigrade. The other potentiometer pole is connected by a constantan wire with a constantan needle which is attached to a sliding contact moving over the copper bar and may be pressed down on it at any desired point. To make a melting-point determination, the



(*Courtesy of Eimer and Amend.*)

FIG. 271. Dennis's melting-point apparatus.

heating element is set in operation and a small amount of the substance to be tested is sprinkled on the heated bar. When the proper temperature is reached some of the particles melt and others do not. The contact needle is pressed down on the dividing line between the two zones, and the temperature is read by turning the slide-wire knob on the potentiometer until the galvanometer needle comes to rest at the 0 point. All corrections necessary when a mercury thermometer is employed are avoided, and the determination can be made in much less time than by any of the older methods.

Optical Activity of Hydrazones and Osazones as a Means of Identification. In addition to melting point the optical activity of hydrazones and osazones is sometimes employed as a means of identification.

Owing to the low solubility of some of the compounds and the high color of some of the solutions the polarization of hydrazones and osazones cannot always be measured with exactness. In hydrazones the

existence of different isomers, discussed on p. 668, may cause wide differences in polarization. The osazones may likewise exist in several isomeric modifications, as ring or chain compounds. Wolfrom, Konigsberg, and Soltzberg[57] have prepared a tetra-acetate of *d*-glucose phenylosazone and established its chain structure. Percival and Percival,[58] on the other hand, have obtained a derivative of *d*-glucose phenylosazone which has a pyranose ring. The structure of the osazones themselves and the mechanism of the mutarotation observed in a number of cases has not been established. The mutarotation may be due to α–β isomerism of the ring compound, or to hydrolysis of the osazones, as observed by Engel,[59] or to a tautomeric change from the hydrazone to the azo form, as suggested by Zerner and Waltuch:[60]

$$\begin{array}{l} CH=N-NHC_6H_5 \\ | \\ C=N-NHC_6H_5 \\ | \end{array} \longrightarrow \quad \begin{array}{l} CH_2-N=NC_6H_5 \\ | \\ CH-N=NC_6H_5 \\ | \end{array}$$

$$\begin{array}{l} CH=N-NHC_6H_5 \\ | \\ C=N-NHC_6H_5 \\ | \end{array} \longleftarrow \quad \begin{array}{l} CH_2-N=NC_6H_5 \\ | \\ CH-N=NC_6H_5 \\ | \end{array}$$

The rotary power of hydrazones and osazones also varies greatly for different solvents. Thus Lobry de Bruyn and van Ekenstein[61] found the following rotations for different β-naphthylhydrazones in methyl alcohol and glacial acetic acid.

	Methyl Alcohol	Glacial Acetic Acid
Rhamnose-β-naphthylhydrazone	+ 8.4	−11.8
Glucose-β-naphthylhydrazone	+40.2	0
Mannose-β-naphthylhydrazone	+16.8	0
Galactose-β-naphthylhydrazone	+24.8	+ 2

For purposes of comparison and identification the rotations of hydrazones and osazones must be measured, therefore, under exactly similar conditions as to quantity of material and nature of solvent. Neuberg[62] recommends dissolving 0.2 g. of osazone in a mixture of 4 ml. pyridine and 6 ml. absolute alcohol, and reading the solution in a 100-mm. tube in a polarimeter. The following rotations were obtained

[57] *J. Am. Chem. Soc.*, **58**, 490 (1936).
[58] *J. Chem. Soc.*, **1935**, 1398.
[59] *J. Am. Chem. Soc.*, **57**, 2419 (1935).
[60] *Monatsh.*, **35**, 1025 (1914).
[61] *Rec. trav. chim.*, **15**, 226 (1896).
[62] *Ber.*, **32**, 3384 (1899).

by Neuberg for different osazones when working under the above conditions:

TABLE XCII

ROTATION OF DIFFERENT OSAZONES

l-Arabinose phenylosazone	+1°10′
l-Arabinose *p*-bromophenylosazone	+0°28′
Xylose phenylosazone	−0°15′
Xylose *p*-bromophenylosazone	±0°
Rhamnose phenylosazone	+1°24′
d-Glucose phenylosazone	−1°30′
d-Glucose *p*-bromophenylosazone	−0°31′
d-Galactose phenylosazone	+0°48′
Sorbose phenylosazone	−0°15′
Maltose phenylosazone	+1°30′
Lactose phenylosazone	±0°

The rotations are small and sometimes uncertain so that this method of identification upon the whole is less satisfactory than a melting-point determination. But with some sugars the initial and final rotations of the phenylosazones or other osazones have been found valuable for identification of the sugar.

For the hydrazones and osazones of optically opposite isomeric sugars (which, as regards melting point and solubility, behave alike except where optically active hydrazines are used), a determination of the optical activity of the compound is the only ready means of identification. Thus Fischer[63] gives for the phenylhydrazones of *d*- and *l*-galactose the following constants.

	Melting Point	$[\alpha]_D$
d-Galactose-phenylhydrazone	158°	−21.6
l-Galactose-phenylhydrazone	158°	+21.6

Fischer also gives for the phenylhydrazones of *d*- and *l*-mannose

	Melting Point	Rotation
d-Mannose-phenylhydrazone	195°	−1.2
l-Mannose-phenylhydrazone	195°	+1.2

The rotations in the latter case were the angular readings obtained in a 100-mm. tube upon a solution of 0.1 g. hydrazone in 1 ml. cold concentrated hydrochloric acid and diluted with 5 ml. of water.

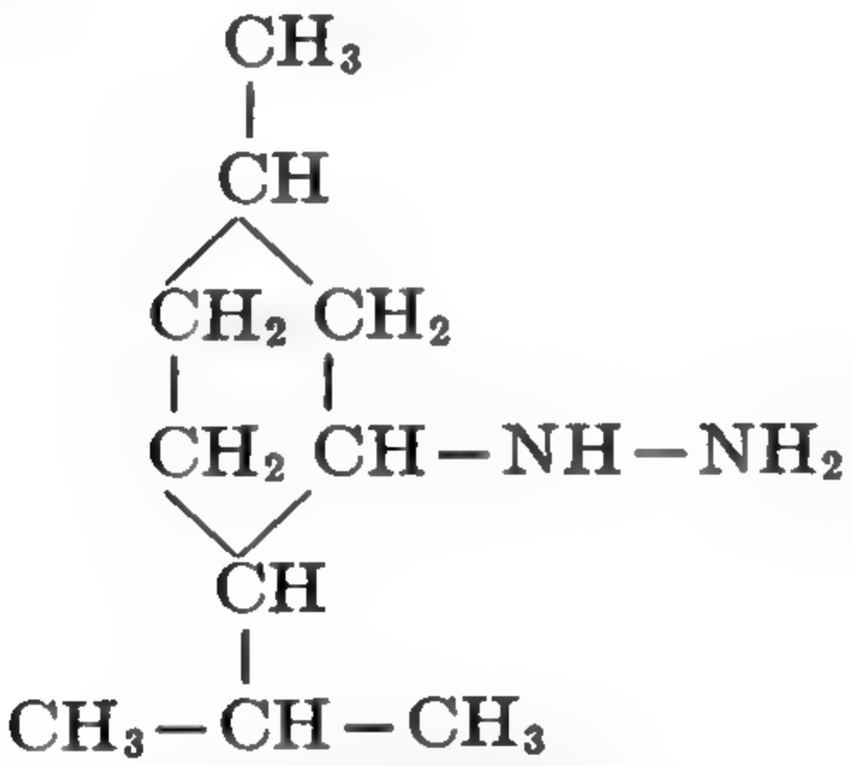
Employment of Optically Active Hydrazines for Separating Sugars from Racemic Mixtures. Neuberg[64] has employed optically active hydrazines for analyzing racemic mixtures of sugars.

[63] Fischer's "Untersuchungen über Kohlenhydrate."
[64] *Ber.*, 36, 1192 (1903); 38, 866, 868 (1905).

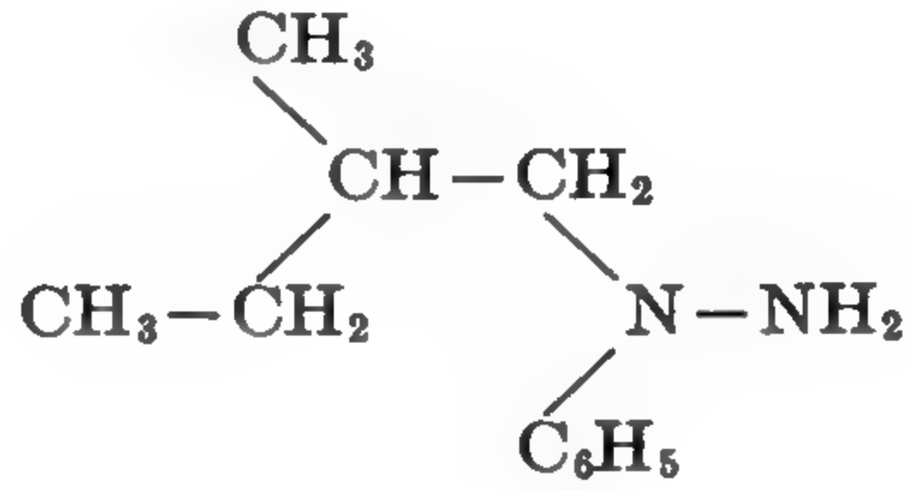
If two optically opposite isomeric sugars ("antipodes") +S and —S form hydrazones with an optically inactive hydrazine H, the resulting compounds, which may be represented by the symbols +SH and —SH, are also antipodes, and, although of exactly opposite rotations, have in other respects, such as specific gravity, melting point, solubility, etc., the same physical properties. A separation of two such hydrazones is consequently not possible by the ordinary methods of analysis.

If, however, the two sugars +S and —S combine with an optically active hydrazine as +H, the resulting hydrazones +S + H and —S + H are not optical antipodes and show well-defined differences in solubility, melting point, and other properties. A separation of the two hydrazones is thus made possible by the ordinary methods of fractional crystallization.

The hydrazines which have been used by Neuberg and his coworkers for this method of separating sugars are *l*-menthylhydrazine and *d*-amylphenylhydrazine, the structural formulas of which are as follows:



l-Menthylhydrazine



d-Amylphenylhydrazine

The method has been employed successfully by Neuberg in resolving the racemic sugar *d,l*-arabinose, which occurs in the urine of many persons suffering from pentosuria; *d,l*-arabinose gives with *l*-menthylhydrazine an easily soluble *l*-arabinose-*l*-menthylhydrazone and a very insoluble *d*-arabinose-*l*-menthylhydrazone. The latter is filtered off and upon treatment with formaldehyde is easily decomposed with liberation of the free sugar *d*-arabinose.

PROPERTIES OF THE HYDRAZONES AND OSAZONES USED FOR THE IDENTIFICATION OF THE MORE COMMON REDUCING SUGARS

Among the large number of condensation products formed from reducing sugars and aromatic hydrazines there are always some which

are characteristic for individual sugars and serve as an aid to their identification. The properties of the principal hydrazones and osazones that may be employed for this purpose are given in the list below, based largely on results obtained by van der Haar,[65] and arranged according to the sugars.

***l*-Arabinose.** α-Benzylphenylhydrazone, colorless needles from 75 per cent alcohol; melting point 174° C.; $[\alpha]_D$ in glacial acetic acid −12.8, in methyl alcohol −12.1. — Diphenylhydrazone, colorless glistening needles from 96 per cent alcohol; m.p. 204° C.; $[\alpha]_D$ in pyridine +14.9. This hydrazone may be employed for the quantitative determination of arabinose (see p. 964). — *p*-Bromophenylhydrazone, yellowish needles from 50 per cent alcohol; m.p. 167 to 168° C.; $[\alpha]_D$ in pyridine −19.9, after 24 hours 0. — α-Methylphenylhydrazone, glistening plates from 30 per cent alcohol; m.p. 165° C. — Benzoylhydrazone, from 95 per cent alcohol, m.p. 207° C. — Diphenylmethane dimethyldihydrazone,[65] precipitated with alcohol from solution in pyridine; amorphous, m.p. 180° C.

The hydrazones of *d*-arabinose are similar to those derived from *l*-arabinose, but show opposite rotation.

***d*-Xylose.** *m*-Nitrophenylhydrazone, yellow glistening needles from 96 per cent alcohol; m.p. 163° C. — Phenylosazone, fine lemon-yellow needles from 30 per cent alcohol; m.p. 163° C.; levorotatory. If much arabinose is present it is precipitated as the α-benzylphenylhydrazone, the filtrate decomposed with formaldehyde, and the xylose identified as the cadmium double salt of xylonic acid and hydrobromic acid (p. 725). β-Naphthylhydrazine may also be used to separate the arabinose as the hydrazone; the hydrazone of the xylose may be recovered from the filtrate by evaporation over concentrated sulfuric acid, and recrystallization from chloroform; m.p. 124° C.; $[\alpha]_D$ in glacial acetic acid +15.8.

***l*-Rhamnose.** β-Naphthylhydrazone, small, colorless plates from 95 per cent alcohol; m.p. 192 to 193° C. — *p*-Nitrophenylhydrazone, orange-yellow crystals from 95 per cent alcohol; m.p. 191–192° C.; $[\alpha]_D$ in pyridine-alcohol mixture, initial −50.3, final −8.5.[65] — *m*-Nitrophenylhydrazone, yellow crystals from 95 per cent alcohol; m.p. 159 to 160° C.; $[\alpha]_D$ initial −21.0, after 26 hours +0.3, final +18.3.[66] — *o*-Nitrophenylhydrazone, yellow needles from 95 per cent alcohol; m.p. 154° C.; $[\alpha]_D$ initial −37.5, final −33.5.[67] — *p*-Tolylhydrazone, from 95 per cent alcohol; m.p. 166° C. — *p*-Bromobenzoylhydrazone, colorless glistening felted needles from 96 per cent alcohol; m.p. 191° C. — Phenylosazone, golden yellow needles in star clusters, from 30 per cent alcohol; m.p. 182° C.; $[\alpha]_D = +94$ in pyridine. — *p*-Bromophenylosazone, lemon-yellow needles from 90 per cent alcohol; m.p. 218° C.

[65] "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide," p. 238, 1920.

[66] von Braun, *Ber.*, 43, 1495 (1910).

[67] Butler and Cretcher, *J. Am. Chem. Soc.*, 53, 4363 (1931).

***l*-Fucose.** *p*-Bromophenylhydrazone, slightly yellowish fine needles, grouped in clusters, from water; m.p. 178° C. — Phenylhydrazone, colorless crystals from 95 per cent alcohol; m.p. 170° C. — α-Methylphenylhydrazone, silvery white prisms in clusters, from 30 per cent alcohol; m.p. 180° C. Diphenylhydrazone, colorless fine needles from 96 per cent alcohol; m.p. 197 to 198° C.; $[\alpha]_D = -15.8$. — β-Naphthylhydrazone, colorless short prisms from 95 per cent alcohol; m.p. 200 to 201° C. — *m*-Nitrophenylhydrazone, orange-yellow long prisms; m.p. 204° C. — *p*-Tolylhydrazone, from 96 per cent alcohol; m.p. 169° C. — Benzoylhydrazone, colorless glistening prisms from 96 per cent alcohol; m.p. 197° C. — *p*-Toluenesulfonylhydrazone;[68] m.p. 174° C.; $[\alpha]_D = -17.0$ in pyridine.

***d*-Fucose (Rhodeose).** *p*-Bromophenylhydrazone, m.p. 184° C. — *p*-Toluenesulfonylhydrazone;[68] m.p. 175° C.; $[\alpha]_D = +17.1$. — Methylphenylhydrazone, m.p. 181° C. — Diphenylhydrazone, m.p. 199° C. — Benzylphenylhydrazone, m.p. 178 to 179° C.; $[\alpha]_D = -14.9$ in methyl alcohol.

***d*-Ribose.** *p*-Bromophenylhydrazone, m.p. 164 to 165° C.; $[\alpha]_D$ in alcohol $= -5.7$. — Benzylphenylhydrazone, m.p. 127 to 128° C. — *p*-Chlorobenzylphenylhydrazone, m.p. 144 to 145° C.; $[\alpha]_D$ initial $= -32.7$, final -17.2 in methyl alcohol. — Diphenylmethane dimethyldihydrazone, m.p. 141 to 142° C.

***d*-Apiose.** Benzylphenylhydrazone, from benzene, m.p. 137 to 138° C.; $\alpha^{25}_{579} = -78.5$.[69] — Phenylosazone, yellow needles, m.p. 155 to 157° C. — *p*-Bromophenylosazone, yellow needles from alcohol, m.p. 212° C.

***d*-Glucose.** *p*-Nitrophenylhydrazone, orange-yellow flat prisms from 95 per cent alcohol, m.p. 190° C.; from water long platelets, m.p. 189° C.; from glacial acetic acid, m.p. 195° C.; $[\alpha]_D$ +21.5 in equal parts of pyridine and alcohol. — *p*-Bromobenzoylhydrazone, fine needles from 96 per cent alcohol, m.p. 201° C. — Phenylosazone, light yellow needles from 70 per cent alcohol; m.p. 210° C.; $[\alpha]_D = -50$ in absolute alcohol. — *p*-Bromophenylosazone, fine lemon-yellow needles from 90 per cent alcohol, m.p. 222° C.; levorotatory. — *p*-Nitrophenylosazone, red, by precipitation from pyridine solution with ether; m.p. 257° C.; $[\alpha]_D$ −21.4 in pyridine-alcohol mixture.

***d*-Galactose.** *o*-Tolylhydrazone, colorless glistening needles from 95 per cent alcohol, m.p. 176° C.; this hydrazone is very characteristic for *d*-galactose and useful for its identification. — *m*-Tolylhydrazone, needles from 95 per cent alcohol, m.p. 154° C.; also characteristic for *d*-galactose. — Phenylosazone, golden yellow aggregates of needles from 30 per cent alcohol; m.p. 183 to 188° C.; dextrorotatory in a mixture of pyridine and alcohol, slightly levorotatory in glacial acetic acid. — α-Methylphenylhydrazone, glossy broad needles from 30 per cent alcohol; m.p. 190–191° C.; shows no optical rotation. — *p*-Nitrophenylhydrazone, yellow needles and plates, arranged in clusters, from 95 per cent alcohol; m.p. 194° C., from water m.p. 196 to 197° C.;

[68] Freudenberg and Raschig, *Ber.*, **62**, 373 (1929).
[69] Schmidt, *Ann.*, **483**, 115 (1930).

$[\alpha]_D = +45.6$. — Benzoylhydrazone, colorless prisms from 95 per cent alcohol, m.p. 192 to 193° C.

***d*-Mannose.** Phenylhydrazone, colorless crystals from 60 per cent alcohol; m.p. 199° C.; $[\alpha]_D$ in pyridine, initial +26.6, after 9 hours +6.3, final +33.8.[70] This hydrazone may be used for the quantitative estimation of mannose. — *p*-Tolylhydrazone, from 96 per cent alcohol, m.p. 190 to 191° C.; weakly dextrorotatory. — *p*-Bromophenylhydrazone, colorless rhombs from 50 per cent alcohol; m.p. 208° C. — α-Methylphenylhydrazone, colorless plates from 30 per cent alcohol; m.p. 181° C.; $[\alpha]_D$ in methyl alcohol +8.6. — *p*-Nitrophenylhydrazone, light yellow rhombs from 95 per cent alcohol; m.p. 202 to 203° C.; $[\alpha]_D$ in mixture of pyridine and alcohol +56.0 (constant).[71] — *m*-Nitrophenylhydrazone, light yellow crystals from 95 per cent alcohol; m.p. 162 to 163° C.; $[\alpha]_D$ initial +26.5, final −8.3. — *o*-Nitrophenylhydrazone, orange-red crystals from 95 per cent alcohol; m.p. 172 to 173° C.; $[\alpha]_D$ +52.0 (constant). — Phenylosazone, identical with glucosazone.

***d*-Fructose.** *p*-Nitrophenylhydrazone, yellow prismatic needles from 95 per cent alcohol; m.p. 180 to 181° C.; $[\alpha]_D$ in pyridine and alcohol mixture +16. — *o*-Nitrophenylhydrazone, lemon-yellow needles from 95 per cent alcohol; m.p. 156 to 157° C.; $[\alpha]_D$ in pyridine and alcohol mixture +31. —2,4-Dichlorophenylhydrazone, m.p. 120° C.[72] — 2,4,6-Trichlorophenylhydrazone, m.p. 155° C.[72] — Phenylosazone, identical with glucosazone. — *p*-Nitrophenylosazone, also probably identical with the corresponding glucose compound. — α-Methylphenylosazone, yellow plates from 10 per cent alcohol; m.p. 160 to 161° C.; this osazone is used for the quantitative determination of fructose (see p. 965).

***l*-Sorbose.** Phenylosazone, m.p. 164° C. — *p*-Bromophenylosazone, m.p. 181° C. — *o*-Nitrophenylosazone, m.p. 211 to 212° C. — The α-methylphenylosazone, contrary to the corresponding fructose compound, has not been obtained in crystalline form.

***d*-Glucuronic Acid.** *p*-Nitrophenylhydrazone, yellow needles from 95 per cent alcohol; m.p. 224 to 225° C. — *o*-Nitrophenylhydrazone, orange-yellow needles from 95 per cent alcohol; m.p. 174° C. — *p*-Bromobenzoylhydrazone, colorless plates from 95 per cent alcohol; m.p. 127 to 128° C. — *p*-Tolylhydrazone, from 96 per cent alcohol; m.p. 170° C. — *p*-Bromophenylosazone, light yellow crystals from water; m.p. 205 to 208° C.; $[\alpha]_D = -369$. — Barium salt of glucuronic acid *p*-bromophenylosazone; light yellow needles, m.p. 208 to 210° C.

***d*-Galacturonic and *d*-Mannuronic Acids.** A number of phenylhydrazine and *p*-bromophenylhydrazine derivatives of these acids have been prepared by Niemann, Schoeffel, and Link.[73] The most characteristic compound of galacturonic acid is the *p*-bromophenylhydrazine *p*-bromophenylhydrazone

[70] Butler and Cretcher, *J. Am. Chem. Soc.*, **53**, 4358 (1931).
[71] Butler and Cretcher, *J. Am. Chem. Soc.*, **53**, 4363 (1931).
[72] Votoček and Rys, *Collection Czechoslov. Chem. Commun.*, **1**, 346 (1929).
[73] *J. Biol. Chem.*, **101**, 337 (1933).

galacturonate; m.p. 145 to 146° C.; $[\alpha]_D^{22}$ in methyl alcohol, initial +9.0. For mannuronic acid the *p*-bromophenylhydrazone *d*-mannuronolactone is characteristic; m.p. 160° C.; $[\alpha]_D^{22}$ in methyl alcohol, initial +64.5. For the preparation of these compounds the original literature should be consulted. Unlike barium glucuronate, the barium salts of galacturonic and mannuronic acid yield hydrazones, not osazones, with *p*-bromophenylhydrazine.

For methods that may be used to identify the individual components of mixtures consisting of two or more monosaccharides by means of hydrazones and osazones the chemist is referred to van der Haar's monograph on this subject (see p. 677).

Maltose. Phenylosazone, fine pale yellow needles, m.p. 206° C.; $[\alpha]_D$ in pyridine-alcohol mixture +75; it can be separated from glucosazone by extraction with acetone. — *p*-Nitrophenylosazone, red powder, m.p. 261° C. — *p*-Bromophenylosazone, pale yellow needles, m.p. 198° C. — *p*-Iodophenylosazone, m.p. 208° C.; $[\alpha]_D$ in pyridine initial +82.9, final +66.1. — β-Naphthylhydrazone, m.p. 176° C.; $[\alpha]_D$ in methyl alcohol +10.6.

Lactose. Benzylphenylhydrazone, m.p. 128° C.; levorotatory. — Naphthylphenylhydrazone, brown needles, m.p. 203° C. — Allylphenylhydrazone, light yellow needles, m.p. 132° C.; $[\alpha]_D$ in methyl alcohol −14.6. — Phenylosazone, aggregates of fine yellow prisms, m.p. 210 to 212° C.; levorotatory. — *p*-Nitrophenylosazone, red powder, m.p. 258° C.; dissolves in sodium hydroxide solution with blue color.

Melibiose. Phenylhydrazone, pale yellow crystals, m.p. 145° C. — β-Naphthylhydrazone, m.p. 135° C.; $[\alpha]_D$ in methyl alcohol +15.9. — Allylphenylhydrazone, pale yellow crystals, m.p. 197° C.; $[\alpha]_D$ in methyl alcohol +21.2. — Phenylosazone, m.p. 176 to 178° C.; $[\alpha]_D$ in pyridine +43.2. — *p*-Bromophenylosazone, m.p. 182° C.

Gentiobiose. The melting point of the phenylosazone was found by Helferich, Bäuerlein, and Wiegand[74] to be 170 to 173° C., but lower as well as higher values have been reported by others.

Turanose. Phenylosazone, long yellow needles, m.p. 215 to 220° C.

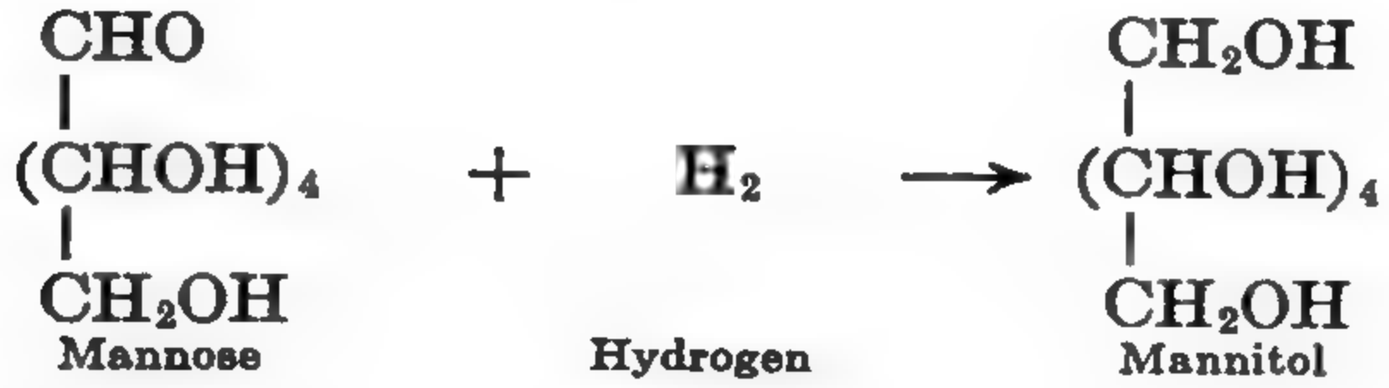
IV. MISCELLANEOUS REACTIONS OF SUGARS[75]

Reactions of Sugars with Reducing Agents. The simple reducing sugars, in their character of aldehydes or ketones, are easily transformed by reducing agents into the corresponding alcohols. The sugar

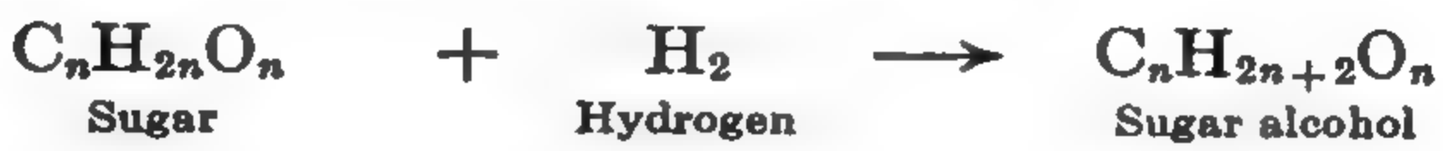
[74] *Ann.*, **447**, 27 (1926).

[75] In order to save space, simplified chain formulas of the reducing sugars are used in most of the equations illustrating the reactions.

mannose, for example, is reduced by sodium amalgam to the alcohol mannitol.

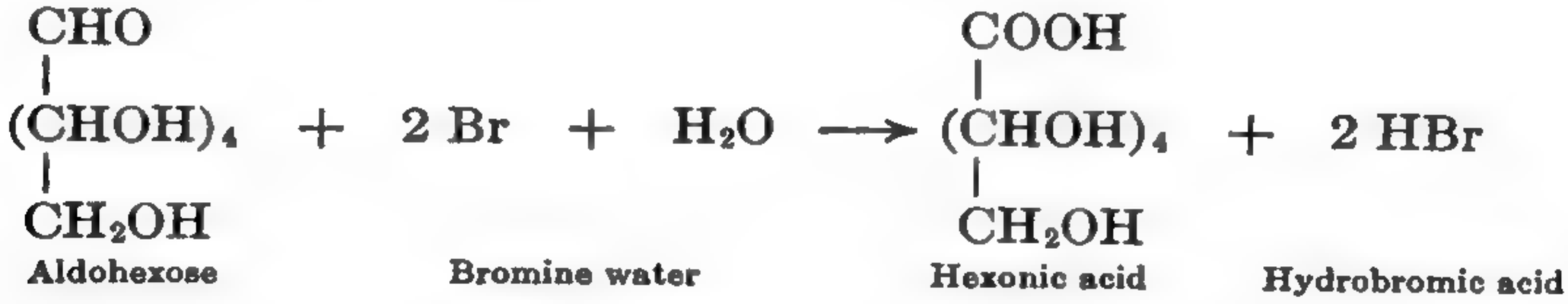


A more general type of equation would be:



Hydrogenation with the aid of catalysts, and electrolytic reduction, are used for the technical preparation of sugar alcohols.

Reactions of Sugars with Weak Oxidizing Agents. Reducing sugars belonging to the aldoses are changed by means of the less powerful oxidizing agents, such as bromine water, into the corresponding monobasic acids. Thus:

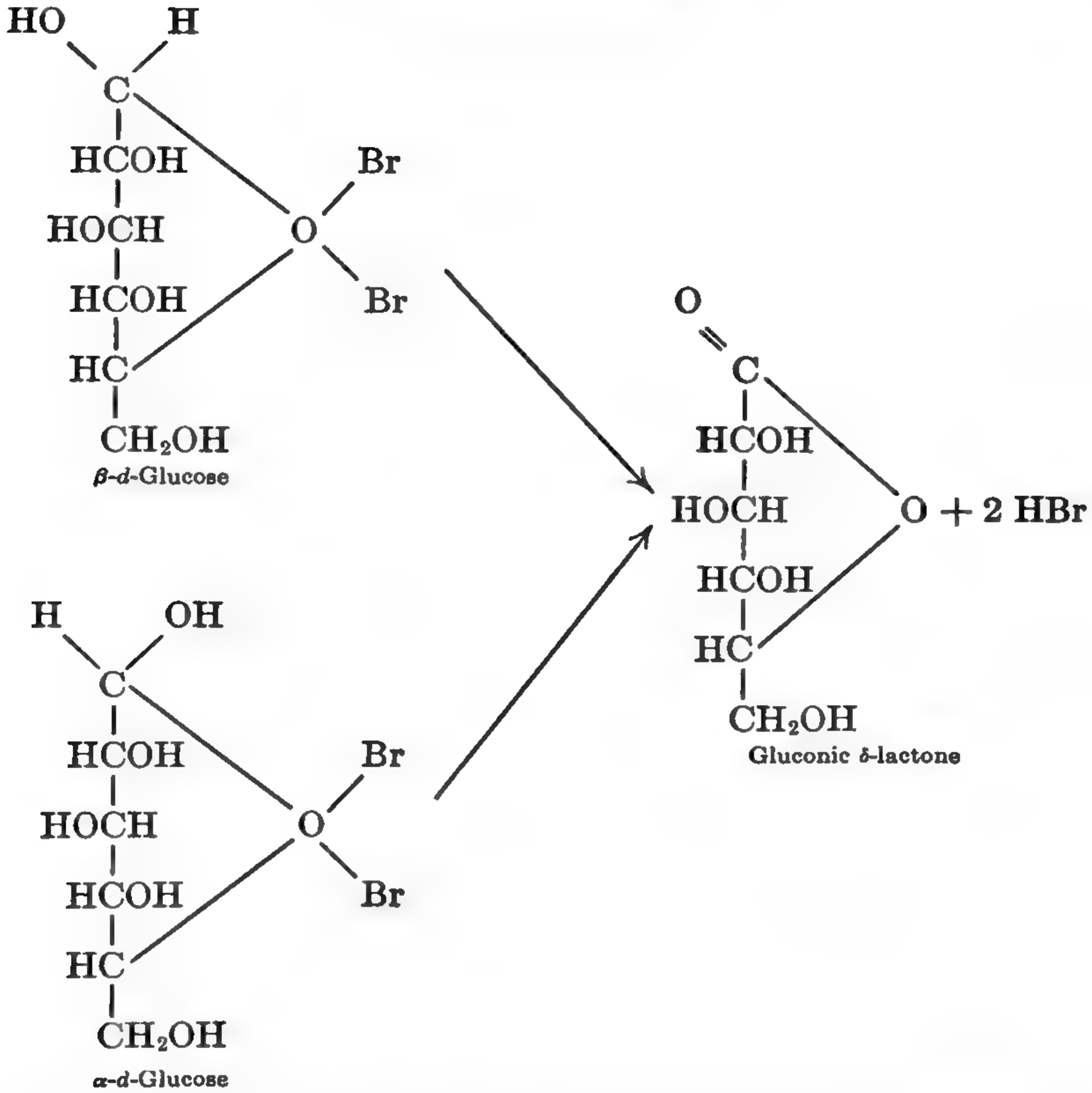


In carrying out the reaction 1 part sugar is treated with 5 parts of water and 2 parts of bromine, and the solution kept at room temperature for 1 to 3 days.

The free acids are unstable and are, upon standing of their solutions or more quickly upon evaporation, partly or completely converted into the corresponding γ or δ lactones.

Isbell and Pigman,[76] by carrying out the oxidation with bromine in a solution saturated with carbon dioxide and in the presence of barium carbonate, found that under these conditions β-*d*-glucose is oxidized 35 times as rapidly as the α-form. In explanation of this they suggest that the glucose first forms an addition product with the bromine. In the case of β-glucose the bromine is closer to the hydrogen on carbon 1 than in that of the α-form, and this facilitates the formation of hydrobromic acid and of gluconic lactone, as may be seen from the following formulas:

[76] *Bur. Standards J. Research*, 10, 337 (1933).



Ketose sugars, upon treatment with bromine water, undergo but little oxidation during the first few days. Prolonged action, or elevation of temperature, will, however, oxidize ketoses with a breaking up of the molecule into several acids of fewer carbon atoms.

Rate of Oxidation with Bromine as a Test for Aldoses and Ketoses. The rate of oxidation of several aldose sugars with bromine water, as compared with fructose, is shown in the following experiments by Votoček and Němeček;[77] 0.5 g. of pure sugar was dissolved in a 50-ml. flask in 9 ml. of water, 40 ml. of bromine water (saturated at room temperature) was then added and the volume made up to 50 ml. After standing at room temperature (21° C.) for 24 hours, the unoxidized sugar was determined in each flask with the following results:

[77] *Z. Zuckerind. Böhmen,* 34, 399 (1909/10).

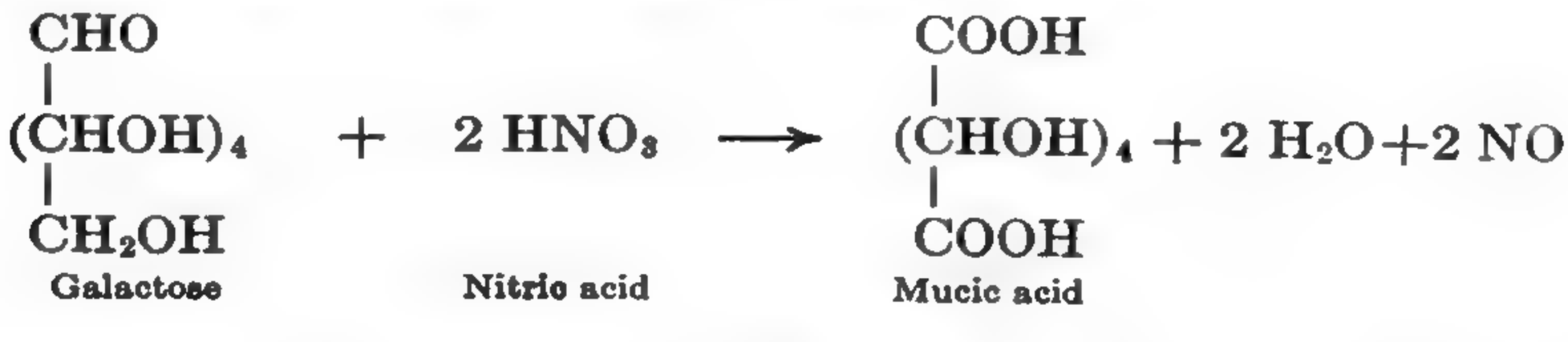
Sugar	Per Cent Sugar Unoxidized	Sugar	Per Cent Sugar Unoxidized
d-Galactose	5.10	*d*-Xylose	25.68
l-Arabinose	7.56	Rhamnose	39.19
d-Glucose	22.20	*d*-Fructose	100.00

Votoček and Němeček propose their method as a means for distinguishing aldoses from ketoses and also as a method for examining sugar mixtures. In case of the latter the aldoses are oxidized away with bromine water, leaving the ketoses in better condition for isolation.

Berg's Reaction for Aldoses.[78] About 20 to 30 mg. of sample is mixed with 10 ml. of freshly prepared bromine water, heated in a water bath to 60–70° C., and the excess bromine is rapidly removed by boiling. Then 10 ml. of a dilute ferric chloride solution (4 drops of a 75 per cent solution of $FeCl_3 \cdot 6H_2O$ and 100 ml. of water) and 2 drops of concentrated hydrochloric acid are added. In the presence of aldoses the aldonic acid formed gives an intense yellow color with the ferric chloride solution. Ketoses give only a faint yellow or no coloration.

Willstätter and Schudel[79] found that under certain conditions iodine in the presence of alkali oxidizes aldoses quantitatively to hexonic acids, while ketoses are not attacked. This reaction has been made the basis for the quantitative determination of aldoses in the presence of ketoses, and of ketoses after complete oxidation of the aldoses. These methods are described in Chapter XV.

Reactions of Sugars with Strong Oxidizing Agents. Reducing sugars belonging to the normal unsubstituted aldoses are changed upon warming with stronger oxidizing agents, as 30 per cent nitric acid, into the corresponding dibasic acids. Thus



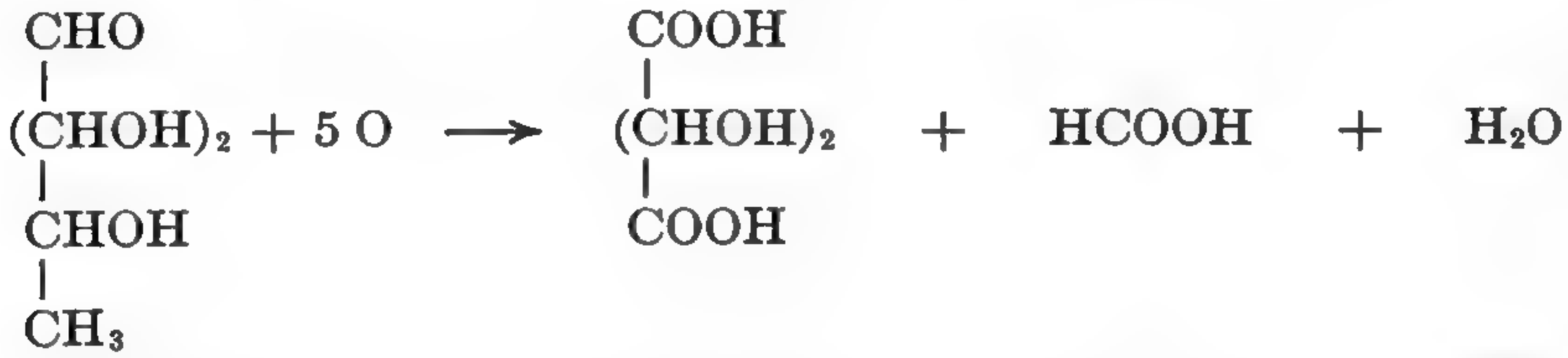
In carrying out the reaction 1 part of sugar is heated with 2½ parts nitric acid of 1.2 sp. gr. and gently warmed at 40° to 50° C. until no more nitrous fumes are evolved. The solution is heated upon the water bath until all nitric acid is expelled and then evaporated, when the acid or its lactone will in many cases crystallize; when crystallization

[78] *Bull. soc. chim.*, [3] 31, 1216 (1904).
[79] *Ber.*, 51, 780 (1918).

does not occur separation from impurities is effected by forming an insoluble salt or other derivative from which the acid can afterward be liberated in the pure condition.

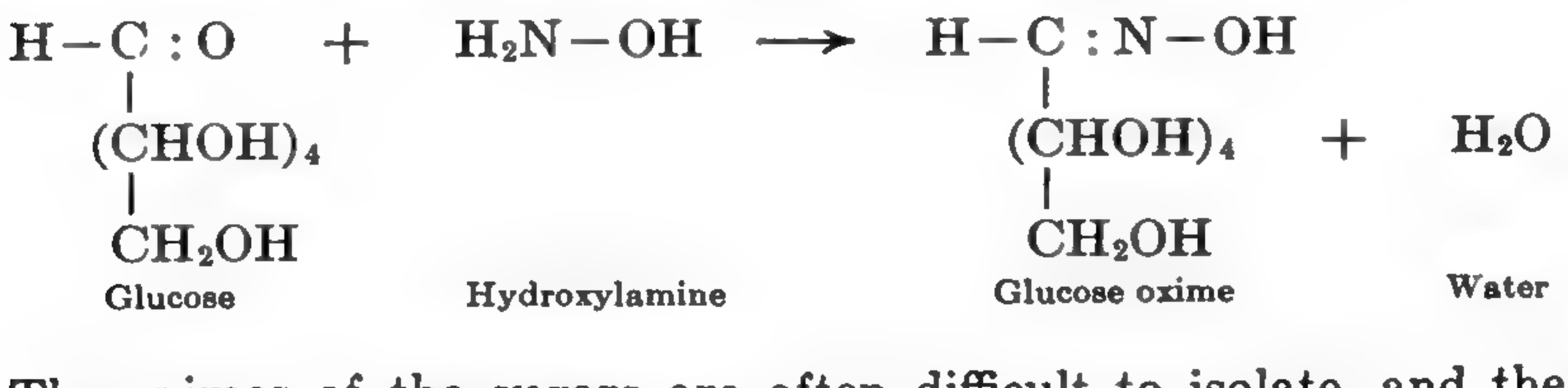
Ketose sugars, upon oxidation with nitric acid, are degraded into lower oxidation products, of which oxalic acid is usually formed in largest amount. Milder treatment with nitric acid splits the chain at the carbonyl, and in the case of fructose yields formic and trioxyglutaric acids.[80]

The substituted aldose sugars, as the methyltetroses, methylpentoses, methylhexoses, etc., lose the methyl group upon oxidation with nitric acid and are degraded into dibasic acids of one less carbon atom.



In the same way the methylpentoses, rhamnose, rhodeose, and fucose, are oxidized into trioxyglutaric acids, the methylhexoses into tetraoxyadipic acids, etc.

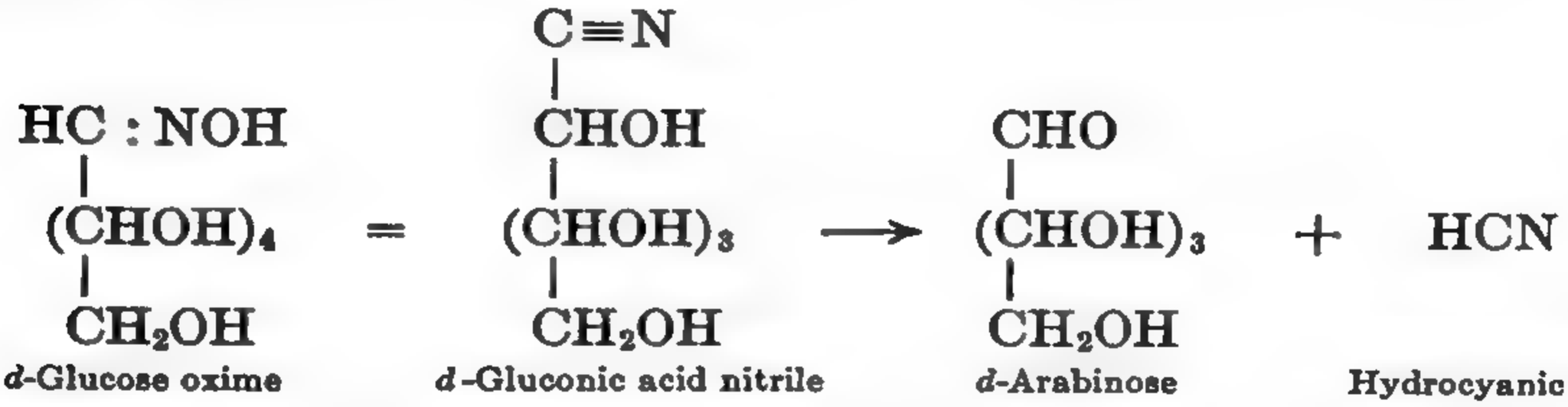
Oxime Reaction of Sugars. Many of the reducing sugars react with hydroxylamine, after the manner of all aldehydes and ketones, with formation of oximes. The following combination of glucose with hydroxylamine is an illustration of this type of reactions.



The oximes of the sugars are often difficult to isolate, and the reaction, for this reason, has but little value in sugar analysis. In sugar synthesis, however, the oxime reaction has considerable importance, for by its means a monosaccharide may be changed into another sugar containing one less carbon atom. This is done by first making the oxime of the sugar and then heating the oxime with acetic anhydride; the resulting acetyl-nitrile derivative is then heated with an ammoniacal solution of silver oxide which splits off the acetic acid and hydrocyanic

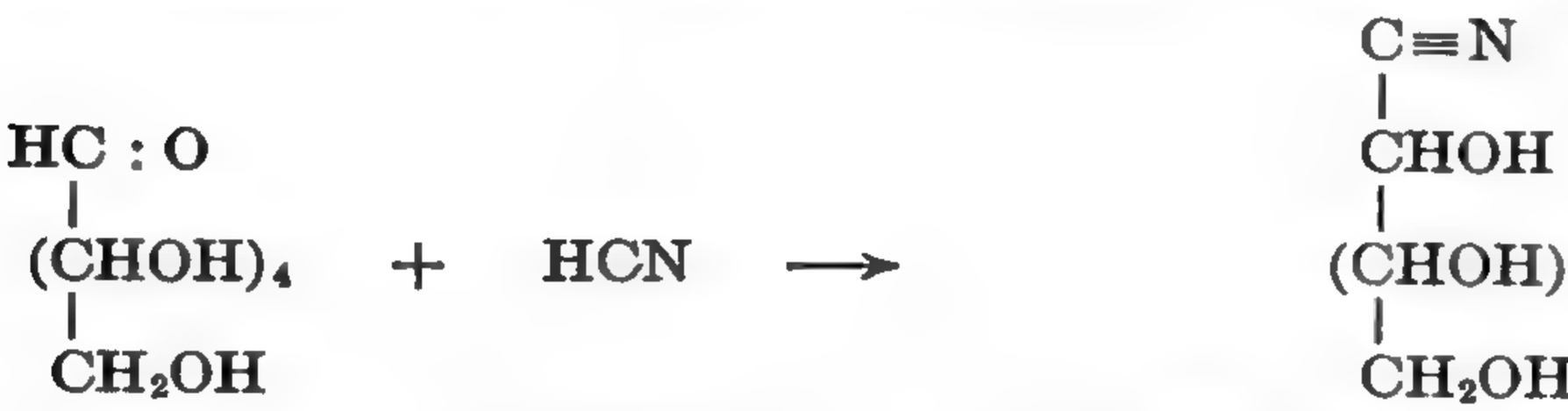
[80] Tollens, " Handbuch der Kohlenhydrate," 3rd ed., p. 315, 1914.

acid groups with formation of a lower sugar (Wohl's[81] synthesis). Much better yields are obtained by saponifying the nitrile with sodium methylate in chloroform.[82] The reaction in its simplest phase is represented as follows:

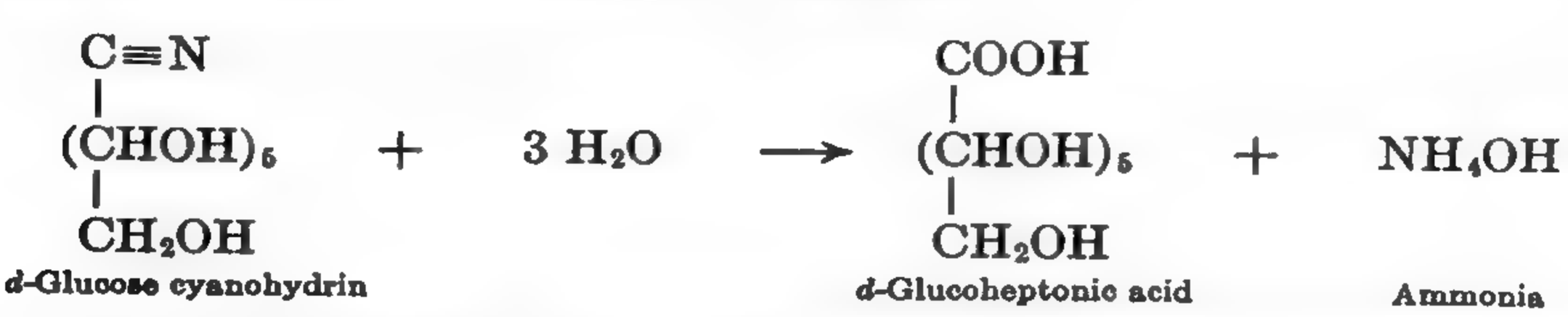


The hexose sugar *d*-glucose is thus converted into the pentose sugar *d*-arabinose. In the same manner *d*-arabinose can be converted into the tetrose sugar *d*-erythrose.

Cyanohydrin Reaction of Sugars. The reducing sugars, similar to all aldehydes and ketones, react with hydrocyanic acid forming a characteristic group of compounds known as cyanohydrins.

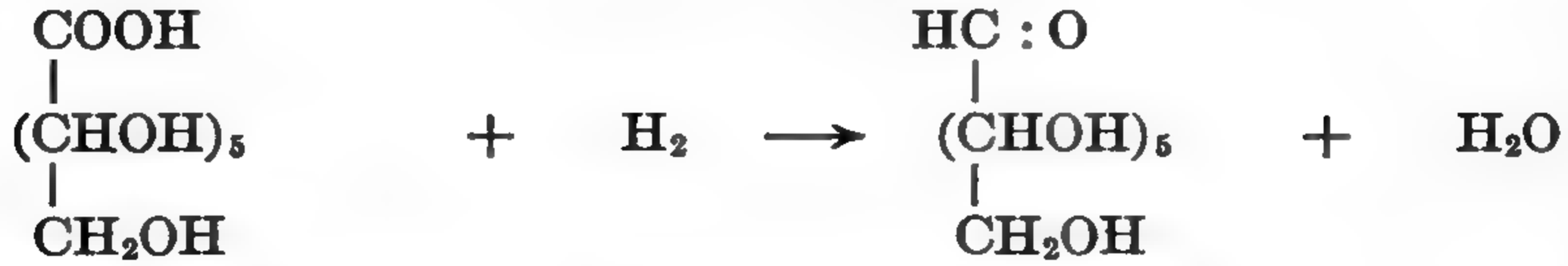


The cyanohydrin reaction, as that of the oximes, while having but little value in sugar analysis, has very great importance in sugar synthesis for by its means a monosaccharide may be built up into another sugar having one more carbon atom. This is done by first making the cyanohydrin, saponifying this to form the corresponding acid, and then reducing the latter with sodium amalgam which produces the corresponding sugar. The formation of glucoheptose from glucose is given as an illustration of this type of reaction.



[81] *Ber.*, **26**, 730 (1893). For the mechanism of the reaction see Wolfrom and Thompson, *J. Am. Chem. Soc.*, **53**, 622 (1931).

[82] Zemplén, *Ber.*, **59**, 1254 (1926); **60**, 165 (1927).



d-Glucoheptonic acid (lactone) — Hydrogen — *d*-Glucoheptose — Water

In the same manner, starting from the hexoses, mannose and galactose, mannoheptose and galaheptose can be derived. The heptoses by the same cyanohydrin synthesis have been built up into the corresponding octoses $C_8H_{16}O_8$ and the latter in turn into the corresponding nonoses $C_9H_{18}O_9$. For details as to this method of forming sugars the work of Fischer[83] should be consulted.

Lippich[84] has applied the cyanohydrin reaction to the identification of reducing sugars. The apparatus used consists of a round flask with a reflux condenser the upper end of which is connected with two absorption flasks containing 35 and 15 ml. respectively of 10 per cent potassium hydroxide solution. The round flask is also provided with a glass tube reaching to the bottom of the flask through which air free from carbon dioxide is drawn by means of an aspirator connected with the exit tube of the second absorption flask. Five grams of the sugar is dissolved in water in a graduated cylinder to a total volume of 90 ml., and the temperature is adjusted to 19° C. The solution is then poured into the round flask in which 20 ml. of 0.25 *N* potassium cyanide solution has previously been placed. The cylinder is washed with 10 ml. of water, and this is also run into the flask. The reaction is allowed to proceed at 19° for exactly 10 minutes. The solution is then quickly acidified with a solution of 40 g. tartaric acid in 70 ml. of water, and the acid solution washed into the flask with another 10 ml. of water. The reflux condenser is then immediately attached, and the uncombined hydrocyanic acid is distilled over into the absorption flask, the air current being adjusted to 30 to 40 bubbles per minute. The distillation is carried on for 2 to 2½ hours, and at the end of that time the hydrocyanic acid in the two absorption flasks is determined by titration according to Liebig's or Volhard's method with standard silver nitrate. The hydrocyanic acid which combined with the sugar is then determined by substracting the number of milliliters found in the distillate from the 20 ml. originally present. This difference is termed by Lippich the " hydrocyanic acid number," which is characteristic for each sugar. Table XCIII shows the results obtained by Lippich with several carbohydrates:

[83] *Ann.*, **270**, 64 (1892); **288**, 139 (1895).
[84] *Z. anal. Chem.*, **76**, 401; **77**, 3 (1929); *Biochem. Z.*, **248**, 280 (1932).

TABLE XCIII

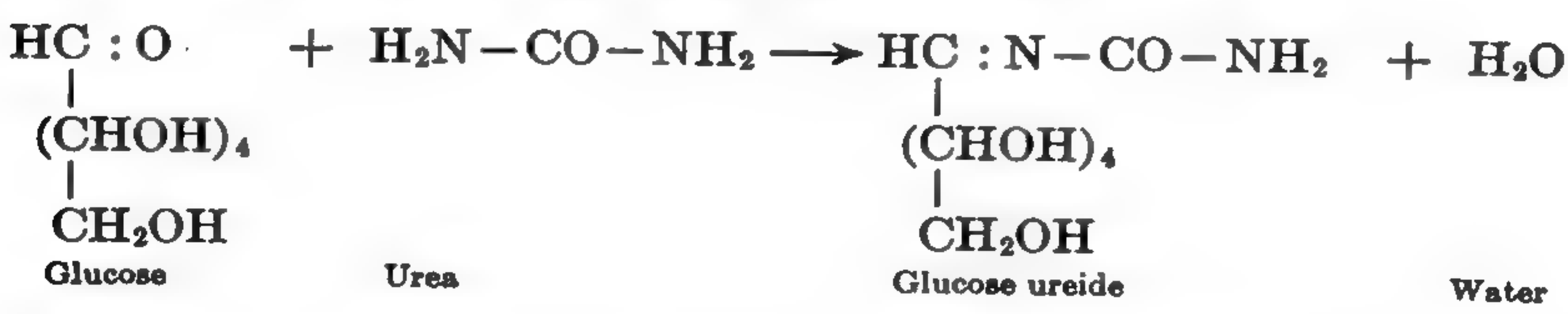
Sugar	Hydrocyanic Acid Number	Hydrocyanic Acid
		grams
Glucose	9.14	0.0240
Fructose	16.77	0.0453
Invert sugar	13.41	0.0362
Lactose	3.54	0.0095
Maltose	5.10	0.0138

Cane sugar and dextrin do not combine with hydrocyanic acid. When more than 5 g. of the sugars enumerated in the table is used, the hydrocyanic acid number does not increase in direct proportion with the sugar concentration, but rises more slowly. For mixtures of sugars the hydrocyanic acid number is not strictly additive, but it is possible to construct families of curves from which the concentration of the constituents can be read off. The application of Lippich's method to sugar mixtures, with and without the inversion procedure in the presence of sucrose, is obvious, but if inversion with acid is practiced, further corrections must be introduced.

The method is too cumbersome to be of much use in practical sugar analysis, but it may prove useful as a further means of identification of individual carbohydrates.

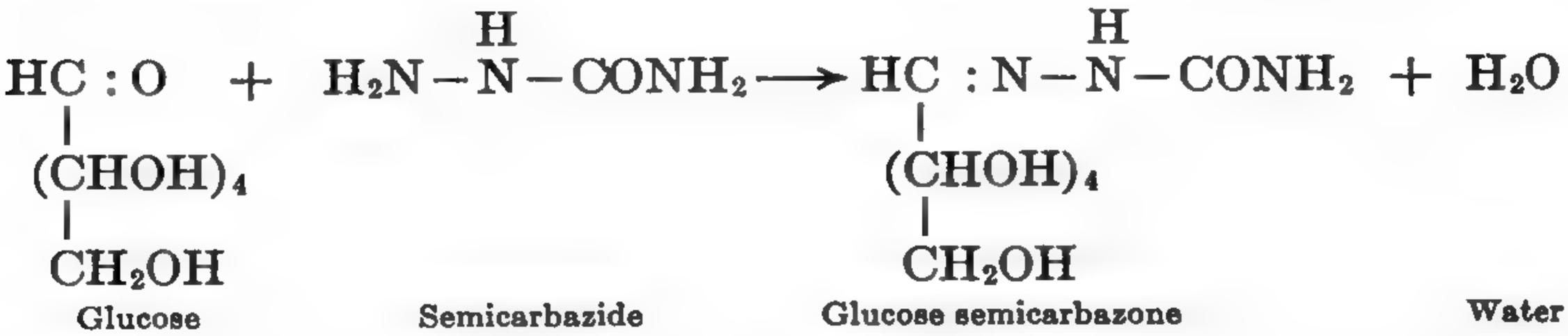
Lippich found later that the capacity of a sugar to combine with hydrocyanic acid is a measure of the aldehydo or keto form in equilibrium with the ring modifications of the sugar.

Ureide Reaction of Sugars. Nearly all reducing sugars, with the exception of the ketoses, react at moderately warm temperatures with urea in presence of dilute sulfuric or hydrochloric acid to form a group of compounds called ureides. The reaction is analogous to that with phenylhydrazine, the hydrogen of the amino group withdrawing the oxygen from the aldehyde group of the sugar. The reaction with glucose and urea is given by way of example.



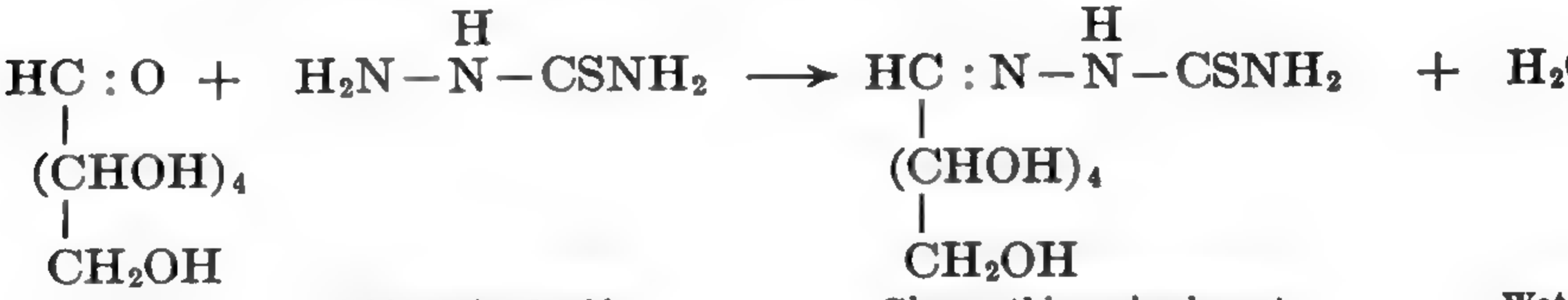
The ureides are partly crystalline and partly amorphous bodies. In aqueous solution they are decomposed upon heating with evolution of ammonia and liberation of the free sugar.

Semicarbazone Reaction of Sugars. Very similar to the reaction of sugars with urea is that with semicarbazide; the latter in alcoholic solution combines with the aldoses to form a group of substances called semicarbazones. The reaction with glucose is given as illustration.



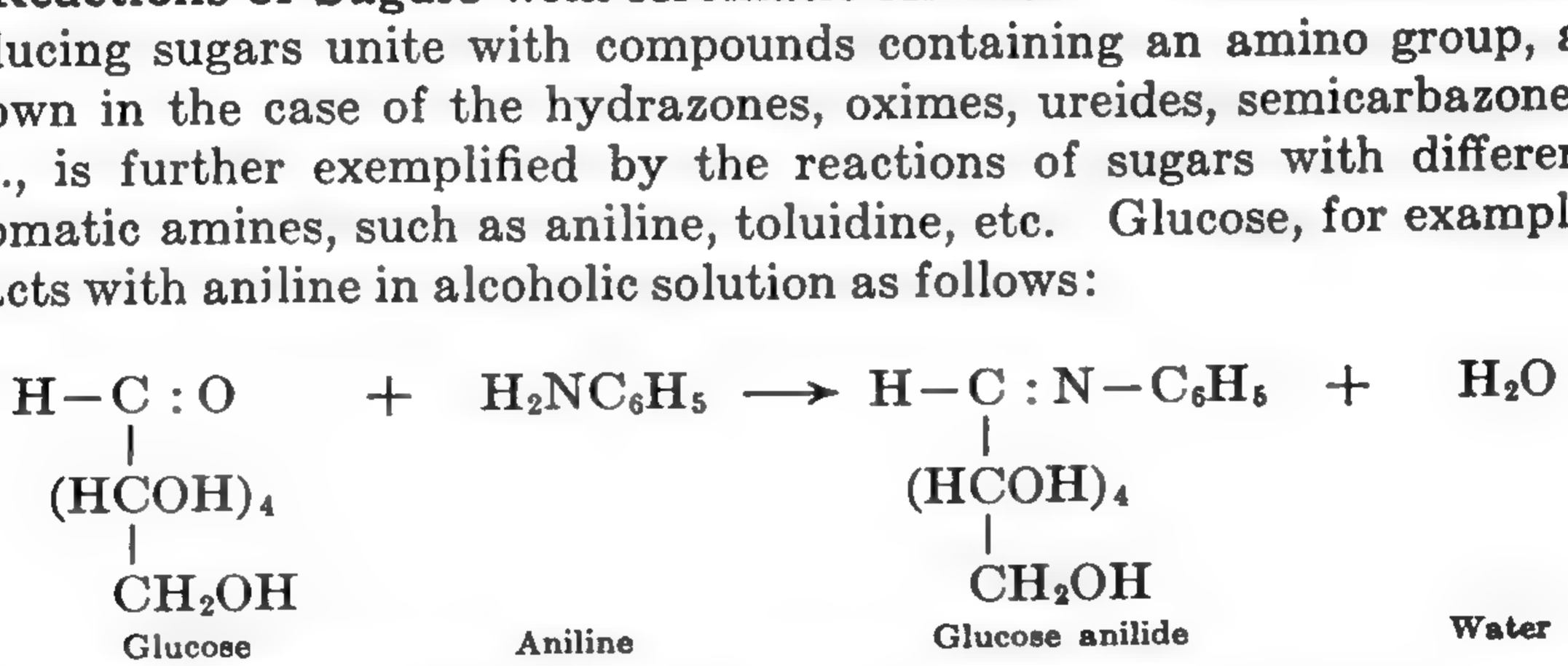
The semicarbazones are well-defined crystalline compounds; when warmed with benzaldehyde in alcohol solution they are decomposed into free sugar with formation of benzaldehyde semicarbazone.

Thiosemicarbazone Reaction of Sugars. Exactly similar to the previous reaction is the behavior of aldose sugars with thiosemicarbazide. The reaction with glucose proceeds as follows:



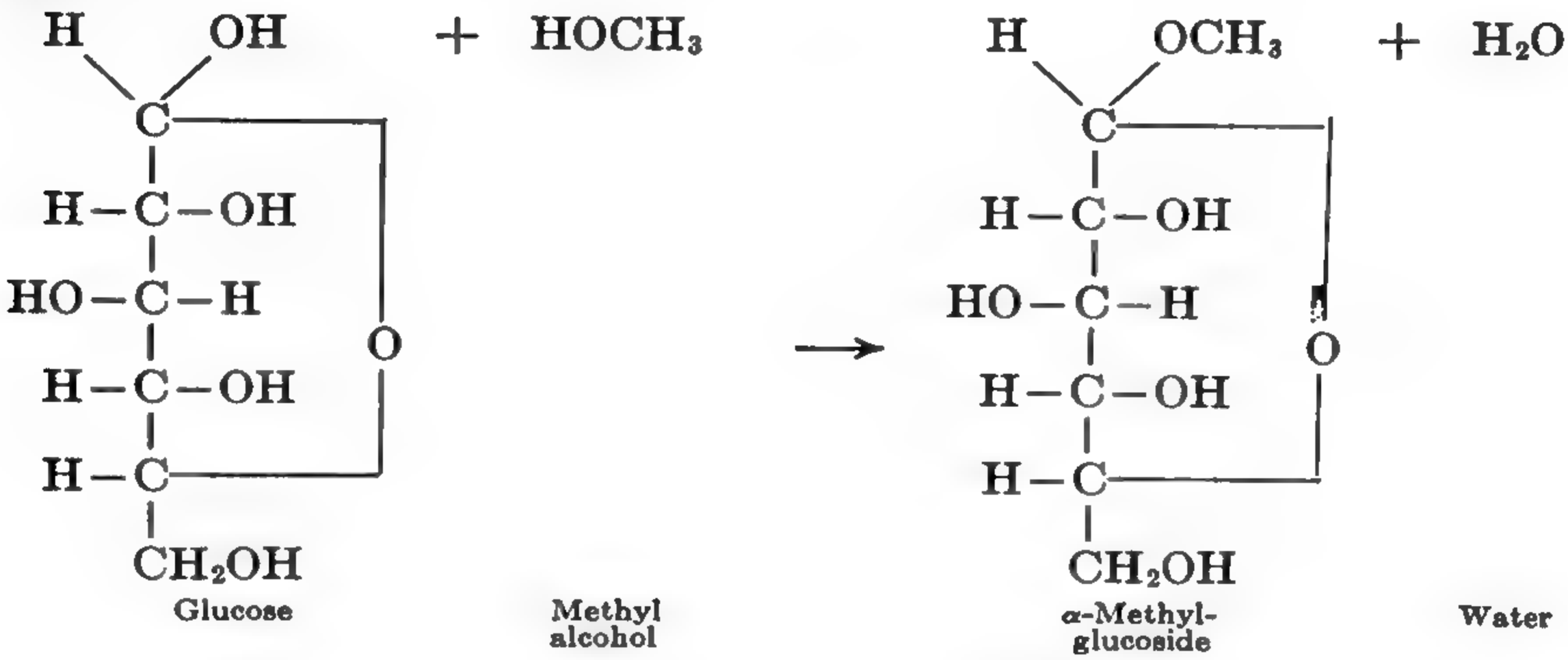
The thiosemicarbazones are well-defined crystalline compounds similar in many properties to the semicarbazones.

Reactions of Sugars with Aromatic Amines. The ease with which reducing sugars unite with compounds containing an amino group, as shown in the case of the hydrazones, oximes, ureides, semicarbazones, etc., is further exemplified by the reactions of sugars with different aromatic amines, such as aniline, toluidine, etc. Glucose, for example, reacts with aniline in alcoholic solution as follows:



Reactions of Sugars with Alcohols. By leading dry hydrochloric acid gas into the solution of a reducing sugar in an alcohol the corresponding alcohol derivative of the sugar is formed. The compounds

thus prepared are called glucosides from their resemblance to the group of plant substances known under this name. The reaction of glucose with methyl alcohol is given as illustration.



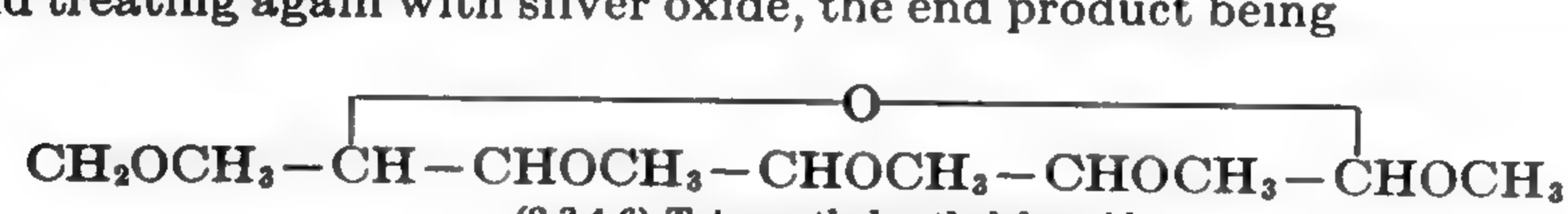
Glucose — Methyl alcohol — α-Methyl-glucoside — Water

In the β-methyl glucoside the methoxyl group is on the side opposite to the oxygen ring. The corresponding glucosides with a 1,4 (furanose) ring instead of the 1,5 (pyranose) ring have also been prepared.

In the same manner glucosides of the other sugars have been made, as methylarabinoside, methylxyloside, methylrhamnoside, methylfructoside, also of the other alcohols as ethylglucoside, etc. The compounds thus prepared are well-defined crystalline substances, easily soluble in water; they do not reduce Fehling's solution and do not react with phenylhydrazine.

The reactions of the reducing sugars with alcohols are but little used as a means of identification. The synthetic glucosides, however, have a great interest for the sugar chemist in other ways.

Additional alkyl groups may be introduced into the simple glucosides.[85] If methylglucoside, dissolved in methyl alcohol, is treated with methyliodide and silver oxide, trimethylmethylglucoside is obtained, and this is further methylated by dissolving it in methyliodide and treating again with silver oxide, the end product being



(2,3,4,6) Tetramethylmethylglucoside

This compound may be made in one step by methylating with dimethyl sulfate and sodium hydroxide.[86] These methyl ethers have

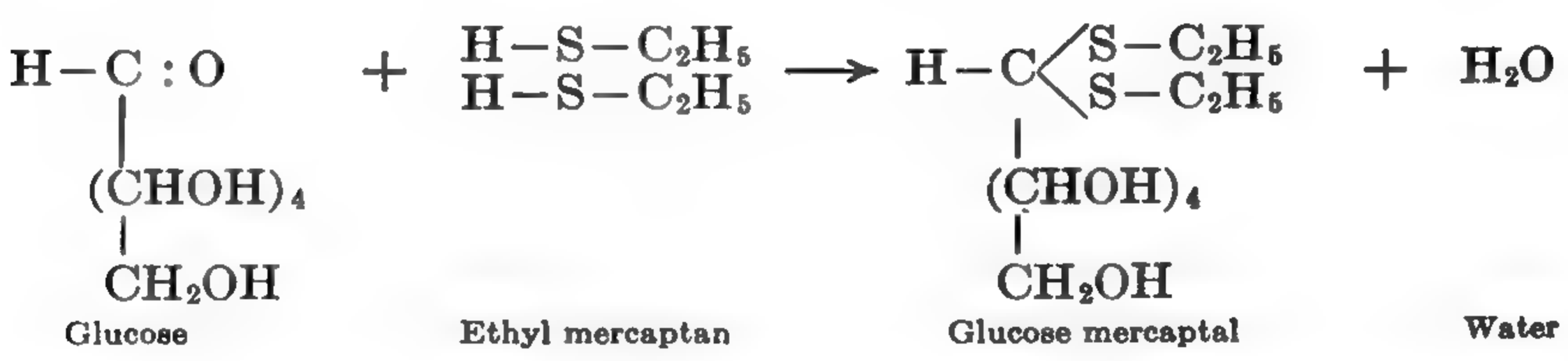
[85] Purdie and Irvine, *J. Chem. Soc.*, 83, 1021 (1903); 85, 1049 (1904).
[86] Haworth, *J. Chem. Soc.*, 107, 8 (1915).

proved of great service in elucidating the structure of sugars and polysaccharides, because the introduction of methyl groups prevents molecular rearrangements which take place easily in the sugars themselves.

The methyl group in glucosidic combination on carbon atom 1 can be split off by hydrolysis with dilute acid, and tetramethylglucose can thus be obtained from tetramethylmethylglucoside. A large number of methyl and ethyl ethers of sugars have been prepared, also some benzyl ethers.

If sugars or sugar derivatives are treated with triphenylmethyl chloride, $(C_6H_5)_3CCl$, in pyridine, the primary alcoholic group of the sugar (e.g., that on carbon 6 in the hexoses) reacts, with the formation of a triphenylmethyl ether. These "trityl" ethers may be used for the identification of sugars. They have proved valuable also for detecting the presence of a primary alcohol group, and as starting material for the synthesis of disaccharides.

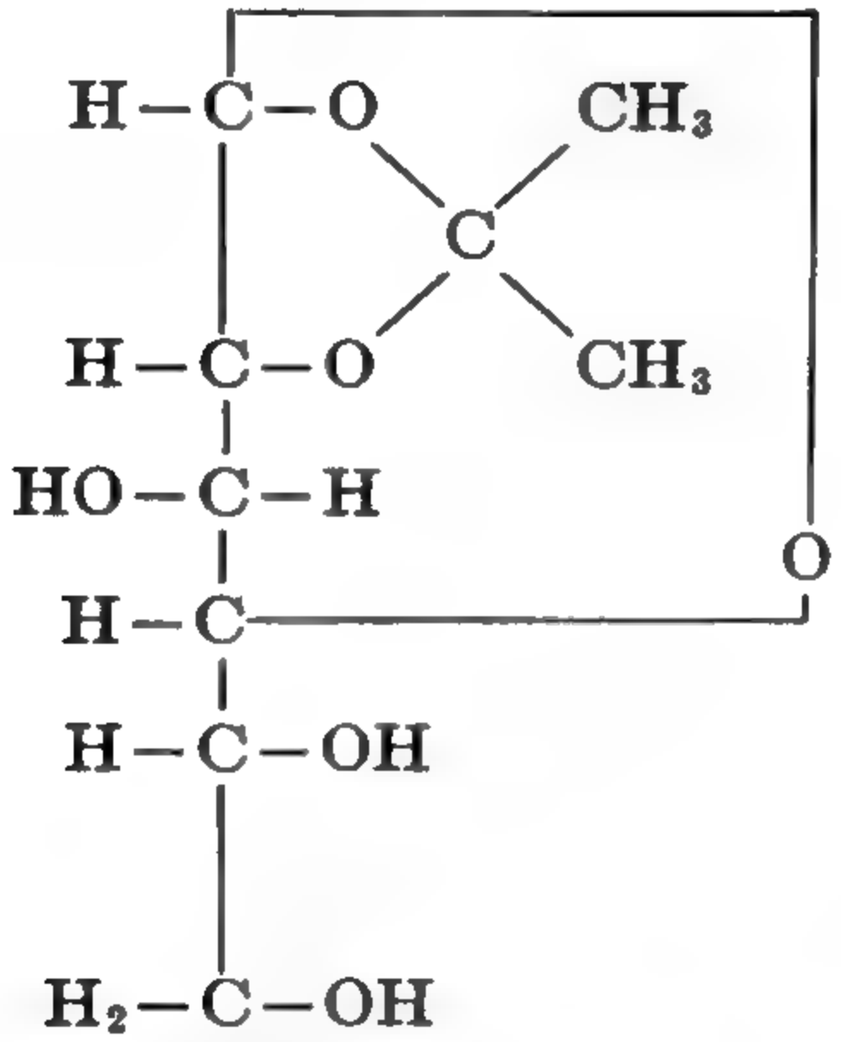
Mercaptal Reaction of Sugars. Nearly all reducing sugars, except ketoses, react with the mercaptans in presence of concentrated hydrochloric acid to form mercaptals. The reaction with glucose and ethyl mercaptan is given as illustration.



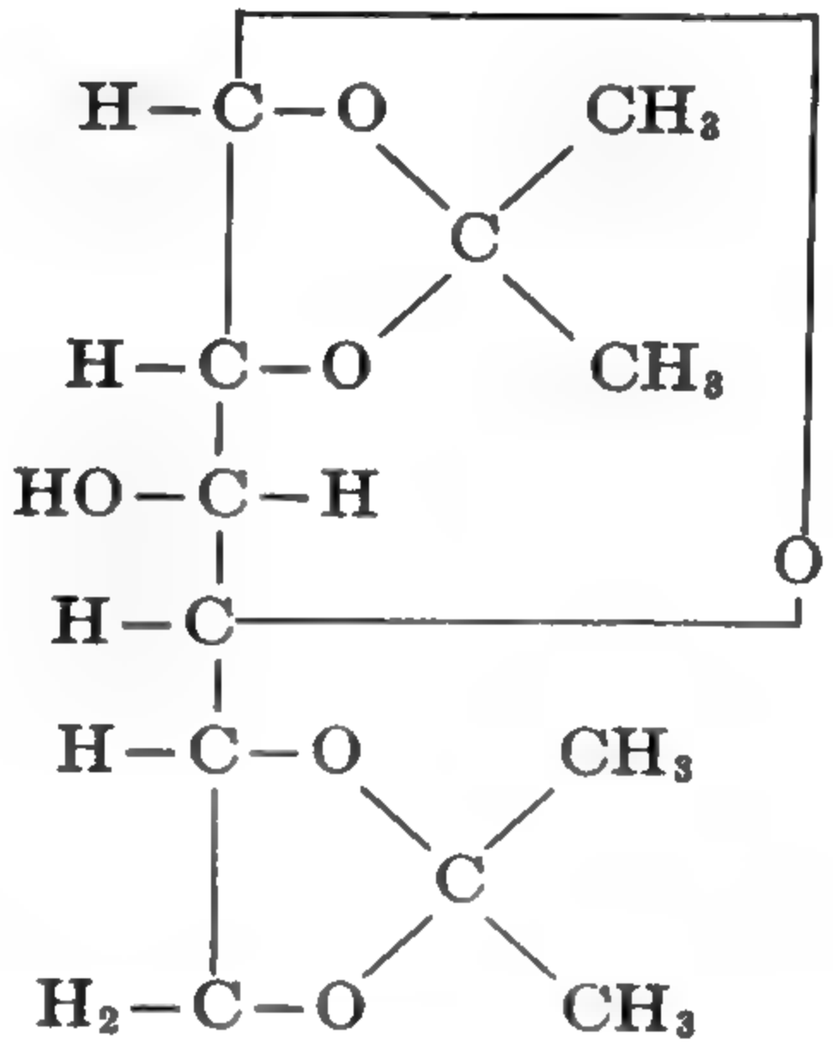
The mercaptals of the sugars are well-defined crystalline compounds, soluble in hot water; they do not reduce Fehling's solution and do not react with phenylhydrazine. The mercaptals are of special interest because they are open-chain compounds and have been found useful for the preparation of other sugar derivatives having this structure.

Reactions of Sugars with Aldehydes and Ketones. The simple reducing sugars react with a large number of aldehydes (formaldehyde, acetaldehyde, benzaldehyde, salicylaldehyde, furfural, etc.) to form a variety of condensation products. Crystalline products have been prepared in which the =CHR group is attached to carbon atoms 1 and 2. In others condensation takes place on carbon atoms 4 and 6, or 5 and 6. In the last instance the compound has the furanose structure. Condensation products of one sugar molecule with two aldehyde molecules have also been prepared. Five such compounds of glucose and chloral are known.

The reducing sugars yield condensation products also with ketones. Those with acetone have played an important role in solving problems of sugar structure. The acetone glucoses are obtained by treating glucose in acetone with a condensing reagent, such as hydrochloric or sulfuric acid, or anhydrous copper sulfate. The following formulas have been assigned to mono- and diacetone glucose, both of which are furanose derivatives:



Monoacetone glucose



Diacetone glucose

Reactions of Sugars with Polyvalent Phenols. The simple reducing sugars unite with different polyvalent phenols (resorcinol, orcinol, hydroquinone, phloroglucinol, pyrogallol, etc.) to form a series of amorphous ill-defined condensation products. The reaction is carried out in the cold in presence of hydrochloric acid. The following combination of arabinose with resorcinol is given as an illustration of this type of reaction.

$$\underset{\text{Arabinose}}{C_5H_{10}O_5} + \underset{\text{Resorcinol}}{C_6H_6O_2} \longrightarrow \underset{\text{Arabinose resorcinol}}{C_{11}H_{14}O_6} + \underset{\text{Water}}{H_2O}$$

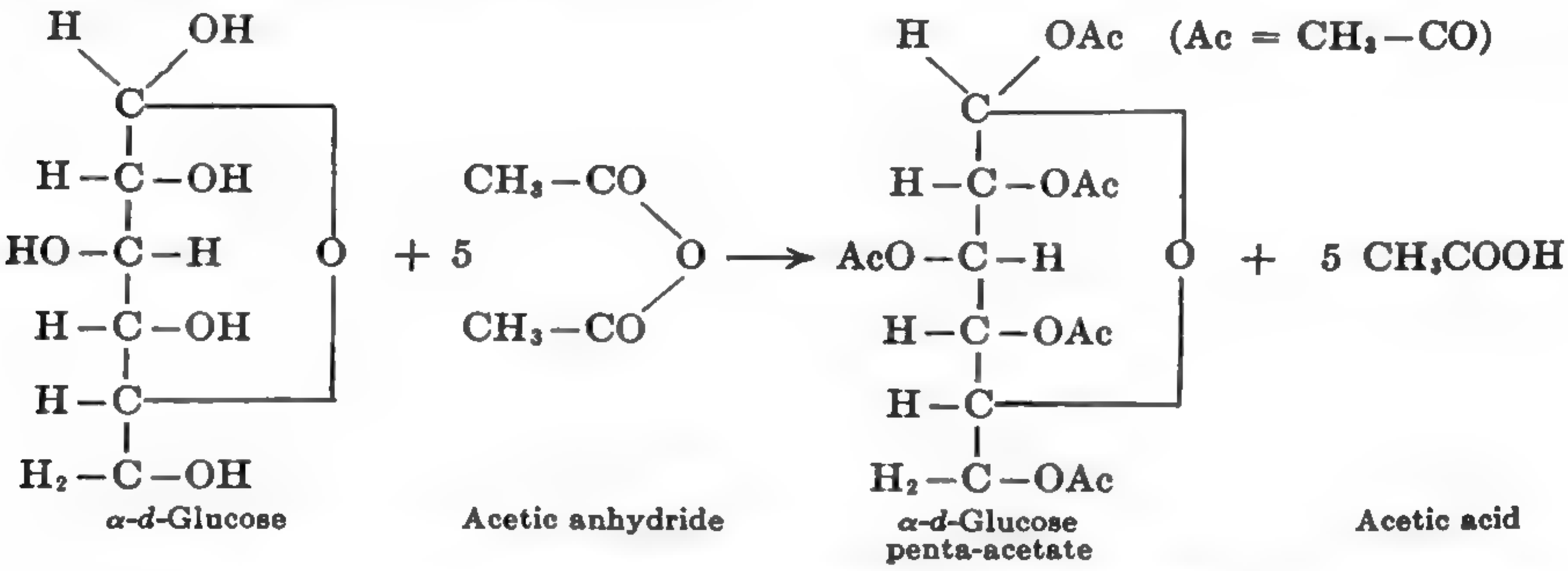
The condensation products of the sugars with polyvalent phenols when heated with concentrated hydrochloric acid are decomposed, showing the color and spectral reactions characteristic for each class of sugar (see p. 659).

True glucosides of various phenols and polyphenols or their derivatives occur in nature, and many have been prepared synthetically.

Reactions of Sugars with Acid Radicals. In most of the reactions previously described the carbonyl group of the sugar molecule is the one primarily affected. There are, however, two types of reactions in

which the alcohol groups are involved. These reactions lead to the formation of ethers or esters. The preparation of ethers has been mentioned on p. 697. The best-known esters prepared in the laboratory are the acetates and benzoates. The number of acid derivatives obtainable with a sugar is dependent upon the number of alcohol groups. In the case of hexoses having five such groups there are mono-, di-, tri-, tetra-, and penta-acetates and benzoates; with sugars of fewer alcohol groups the number of these combinations is correspondingly less.

Reactions of Sugars with Acetic Anhydride. Acetates of the sugars are formed by heating with acetic anhydride. A mixture of acetates usually results during the reaction, the separation of these being effected by fractional crystallization or by the use of different solvents. To obtain completely acetylated products, the reaction must be carried out in the presence of some condensing agent. If glucose is acetylated with the aid of zinc chloride, α-glucose penta-acetate is obtained according to the following formula:



The lower acetates are usually prepared by indirect methods from partially substituted sugars. By saponification of the acetates under mild conditions, as for instance with sodium methylate in the cold, the free sugars are regenerated. The lower acetates reduce Fehling solution, but the fully acetylated sugars do not, neither do they yield hydrazones, oximes, etc.

Acetates with the open-chain structure have been obtained by acetylation of mercaptals and splitting off of the mercaptan.

Reaction of Sugars with Benzoyl Chloride. The acetates of the sugars owing to their solubility are not well adapted for the identification of sugars; the sugar benzoates, however, are marked by a high insolubility in water, and their formation is sometimes used as a qualitative test for sugars.

The test, according to the method of Baumann,[87] is carried out by

[87] *Ber.*, **19**, 3220 (1886).

treating a solution of the sugar with benzoyl chloride in the presence of sodium hydroxide; the benzoic radical displaces the H of the hydroxyl groups with formation of sodium chloride and water. A number of benzoates are usually formed in the reaction. In the case of glucose pentabenzoate the formation proceeds as follows:

$$\begin{array}{l}\mathrm{CHO}\\ |\\ (\mathrm{CHOH})_4\\ |\\ \mathrm{CH_2OH}\end{array} + 5\,\mathrm{C_6H_5COCl} \quad + 5\,\mathrm{NaOH} \longrightarrow \begin{array}{l}\mathrm{CHO}\\ |\\ (\mathrm{CHOCOC_6H_5})_4\\ |\\ \mathrm{CH_2OCOC_6H_5}\end{array} + 5\,\mathrm{NaCl} + 5\,\mathrm{H_2O}$$

Glucose — Benzoyl chloride — Sodium hydroxide — Glucose pentabenzoate — Salt — Water

The Baumann reaction is sufficiently delicate to detect 1 to 2 mg. glucose in 100 ml. of water and is sometimes employed for testing urine; 100 ml. of solution is well shaken with 2 ml. of benzoyl chloride.

According to Fischer and Freudenberg[88] the pentabenzoate is easily prepared by treating glucose with benzoylchloride and pyridine or quinoline, in the presence of chloroform. The esters of a number of organic acids have been made by this method, notably palmitates, stearates, and oleates, whose close relation to the fats is readily apparent.

Many esters of inorganic acids, such at nitric, sulfuric, phosphoric, and boric, are also known, but they are little used for the identification of sugars. Some esters belonging to this group occur in nature, e.g., chondroitin sulfuric acid in cartilage, mucoitin sulfuric acid in mucin and mucoids, etc. Phosphoric acid esters of sugars play an important part in fermentation and also in the utilization of sugars in the animal body.

Special Tests for Reducing Sugars

To the second class of reactions for examining sugars belong the special tests pertaining to group identification; the reactions chosen for description may be divided for convenience into four general classes.

I. Analysis of hydrazones and osazones.
II. Separation of products obtained by decomposition with concentrated hydrochloric acid.
III. Color reactions with phenols in the presence of concentrated mineral acids.
IV. Miscellaneous reactions.

[88] *Ber.*, **45**, 2725 (1912).

I. ANALYSIS OF HYDRAZONES AND OSAZONES AS A MEANS OF IDENTIFYING SUGAR GROUPS

If the hydrazone or osazone of a sugar has been separated in a pure condition, an elementary analysis of the compound will serve to identify the group to which the sugar belongs. The osazones, owing to their greater insolubility and ease of preparation, are best adapted for this purpose. The determinations necessary for the identification of an osazone are those of the elements nitrogen and carbon; a determination of hydrogen is also usually included since this element can be determined with little extra trouble at the same time as the carbon determination.

The elementary analysis of osazones and hydrazones is carried out by burning about 0.2 g. of the substance over cupric oxide in a combustion tube. For nitrogen the combustion is carried out by Dumas's method in a current of carbon dioxide after complete displacement of the air. The evolved nitrogen is received in a eudiometer over strong potassium hydroxide solution and its volume measured. From the volume of gas the weight of nitrogen is calculated, the necessary corrections for atmospheric pressure and temperature being made.

For carbon and hydrogen the combustion is carried out by Liebig's method in a current of air or oxygen which must be perfectly dry and free from carbon dioxide. The evolved water is collected in weighed tubes, or spirals, containing concentrated sulfuric acid, and the evolved carbon dioxide absorbed in weighed Liebig bulbs containing concentrated potassium hydroxide solution, or in U-tubes filled with soda lime ($NaOH + CaO$). From the weights of water and carbon dioxide obtained the percentages of carbon and hydrogen are calculated. The percentage of oxygen in osazones and hydrazones is determined by subtracting the sum of the percentages of the other elements from 100.

In the elementary analysis of osazones and hydrazones, as of all other nitrogen compounds, a spiral of copper should be placed in the combustion tube at the exit end in order to effect the reduction of oxides of nitrogen. For complete details as to methods of combustion the chemist is referred to the standard textbooks upon organic analysis.

The elementary composition of an osazone or hydrazone having been determined, reference to a table of percentage composition will usually locate the class of sugar to which the compound belongs. In the following table the formula and percentage composition of phenylosazones are given for various groups of sugars.

Phenylosazone	Formula	Composition			
		C	H	N	O
		per cent	per cent	per cent	per cent
Diose	$C_{14}H_{14}N_4$	70.54	5.93	23.53	.....
Triose	$C_{15}H_{16}N_4O$	67.12	6.01	20.90	5.97
Tetrose	$C_{16}H_{18}N_4O_2$	64.39	6.08	18.80	10.73
Pentose	$C_{17}H_{20}N_4O_3$	62.16	6.14	17.08	14.62
Methylpentose	$C_{18}H_{22}N_4O_3$	63.12	6.48	16.38	14.02
Hexose	$C_{18}H_{22}N_4O_4$	60.30	6.19	15.64	17.87
Heptose	$C_{19}H_{24}N_4O_5$	58.73	6.23	14.43	20.61
Octose	$C_{20}H_{26}N_4O_6$	57.38	6.27	13.40	22.95
Nonose	$C_{21}H_{28}N_4O_7$	56.22	6.29	12.50	24.99
Disaccharide	$C_{24}H_{32}N_4O_9$	55.35	6.20	10.77	27.68

II. SEPARATION OF PRODUCTS OBTAINED BY DECOMPOSITION WITH CONCENTRATED HYDROCHLORIC ACID AS A MEANS OF IDENTIFYING SUGAR GROUPS

Although an elementary analysis of osazones is one of the best means of determining the class to which a sugar belongs, a number of other special group reactions are of great value. The most important of these is the separation and identification of some characteristic decomposition product obtained by treating the sugar with concentrated sulfuric or hydrochloric acid. The latter acid is less drastic in its action and is the one most commonly used.

The varied nature of the decomposition products — humus substances, aldehydes, acids, etc. — obtained upon heating sugars with concentrated hydrochloric acid has already been mentioned. It is found, however, that when this treatment is carefully controlled some one characteristic decomposition product will predominate for each particular group of sugar. The following equations, representing ideal types of reaction, are given as illustrations:

I. $\underset{\text{Pentose}}{C_5H_{10}O_5} \longrightarrow \underset{\text{Furfural}}{C_5H_4O_2} + \underset{\text{Water}}{3\,H_2O}$

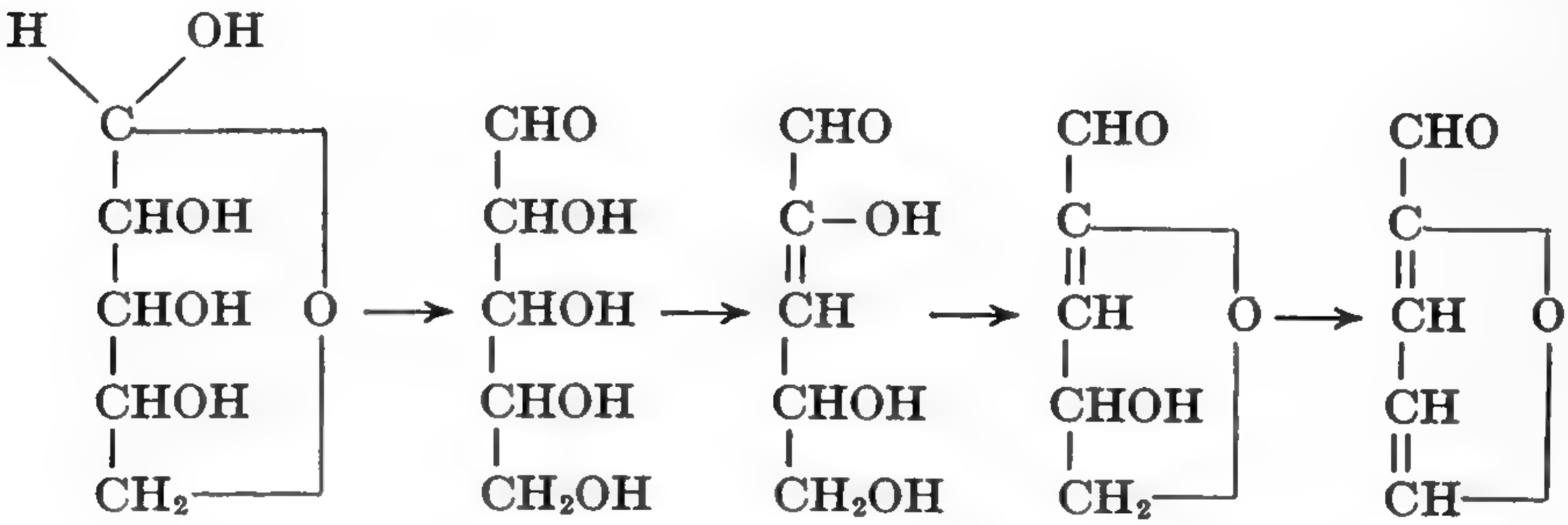
II. $\underset{\text{Methylpentose}}{C_5H_9(CH_3)O_5} \longrightarrow \underset{\text{Methylfurfural}}{C_5H_3(CH_3)O_2} + \underset{\text{Water}}{3\,H_2O}$

III. $\underset{\substack{\text{Hexose}\\\text{(Hydroxymethylpentose)}}}{C_5H_9(CH_2OH)O_5} \longrightarrow \underset{\text{Hydroxymethylfurfural}}{C_5H_3(CH_2OH)O_2} + \underset{\text{Water}}{3\,H_2O}$

The hydroxymethylfurfural formed from hexoses is unstable in the presence of strong acids and decomposes into levulinic and formic acids, according to the equation:

IV. $C_6H_6O_3 + 2\,H_2O \rightarrow \underset{\text{Levulinic acid}}{CH_3-CO-CH_2-CH_2-COOH} + \underset{\text{Formic acid}}{HCOOH}$

The mechanism of the formation of furfural from a pentose is interpreted in this manner by Hurd and Isenhour:[89]



The formation of methylfurfural from a methylpentose, and of hydroxymethylfurfural from a hexose, is perfectly analogous to this rearrangement.

The types of reaction shown in equations I to IV hold true not only of the simple sugars above named but also of the higher saccharides which yield these sugars upon hydrolysis. In fact, the initial phase of the reaction in case of the higher saccharides (sucrose, maltose, lactose, raffinose, starch, pentosans, methylpentosans, etc.) is purely hydrolytic, the simple sugars formed being subsequently decomposed after the manner just indicated.

Levulinic Acid Reaction for Hexose Groups. This reaction, which is due to Tollens[90] and has been extensively studied by his coworkers, has been employed with great success in detecting hexose groups in a large variety of plant and animal substances (cellular tissues of plants, nucleic acids of animal origin, etc.). Owing to the much greater predominance of hexose-producing substances in nature the levulinic acid reaction is usually among the first tests applied in investigating materials of unknown composition.

Description of Test. In carrying out the reaction 5 to 10 g. of material is treated with 20 to 50 ml. of hydrochloric acid of 1.09 to 1.10 sp. gr. (18 to 20 per cent) in a flask provided with a rubber stopper and condensing tube, and heated in a boiling-water bath for 5 to 20 hours. The brownish-colored liquid is then cooled and filtered from the precipitate of humus substances; the filtrate is shaken out in a separatory funnel four times with ether, and the ether extract, after pouring through a dry filter, evaporated. The sirupy residue is then gently

[89] *J. Am. Chem. Soc.*, **54**, 317 (1932).

[90] *Ann.*, **206**, 207, 226 (1881); **243**, 314 (1888); *Ber.*, **33**, 1286 (1900).

heated in an open dish to expel the formic acid (see previous equation IV). If levulinic acid is present a drop of the sirup dissolved in water in the presence of sodium carbonate and iodine will give a precipitate of iodoform, which can also be recognized by its characteristic odor.

The main portion of the sirup is dissolved in water, boiled with an excess of zinc oxide (ZnO), and then, after being decolorized with animal charcoal, filtered and evaporated. The zinc salt of levulinic acid will soon crystallize; the crystals are filtered off, washed with absolute alcohol and ether, and then converted into the silver compound. This is done by dissolving the zinc salt in 5 to 10 ml. of water, adding silver nitrate slightly in excess of the equivalent amount and heating nearly to boiling, with addition of a little water until the precipitated silver salt has completely dissolved. A little animal charcoal is then added and the solution filtered. The levulinate of silver, $C_5H_7O_3Ag$, which crystallizes will show hexagonal crystals or plates under the microscope, in case the compound is pure; if the compound is less pure the crystals will be featherlike in appearance. The silver salt is filtered off, washed with cold water, pressed between filter papers, and dried in a dark place over concentrated sulfuric acid. The percentage of silver in the salt is determined by strongly igniting a weighed portion in a porcelain crucible. The theoretical amount is 48.39 per cent silver.

The yield of levulinic acid obtained by treating hexose sugars with hydrochloric acid will vary greatly according to the time of heating and other conditions of the experiment. Conrad and Guthzeit[91] obtained upon heating 10.5 g. each of fructose, glucose, and galactose, with 50 ml. of acid (containing 4.87 g. HCl gas) for 17 hours the following yield of products:

Sugar	Humus		Levulinic Acid		Formic Acid	
	grams	per cent	grams	per cent	grams	per cent
Fructose...	2.12	20.19	4.09	38.95	1.73	16.48
Glucose....	1.00	9.52	3.12	29.71	1.35	12.86
Galactose..	1.77	16.86	2.85	27.14	1.11	10.57

From these results it appears that of the three hexose sugars fructose gives the largest yield of levulinic acid and galactose the least. That this is due largely to the greater resistance of glucose and galactose toward the acid was shown by the fact that at the end of the above experiments considerable quantities of these sugars were still undecomposed (in case of glucose 26 per cent). The yield of levulinic acid is too variable for the method to be of any quantitative value.

[91] *Ber.*, 19, 2575 (1886).

The levulinic acid reaction is given also by a few sugars not belonging to the hexose group, such as for instance by thyminose (desoxy-*d*-ribose), the sugar of thymonucleic acid.

Furfural Reaction for Pentose Groups. This reaction, which is also due to Tollens,[92] has been of the greatest value not only as a means of detecting the presence of pentose carbohydrates but also as a means of their quantitative estimation.

The reaction of the pentose sugars with hydrochloric acid proceeds much more nearly according to the equation (I, p. 703) than the reaction of the hexoses, the formation of humus substances being correspondingly less.

The theoretical yield of furfural, according to the equation, is 64 per cent; actual determinations of the furfural, obtained by distilling weighed amounts of the pentose sugars, arabinose and xylose, with hydrochloric acid, give about 47 per cent in case of arabinose and about 57 per cent in case of xylose — yields which are about 75 per cent and 90 per cent respectively of the theoretical.

Description of Test. In carrying out the qualitative test about 5 g. of substance is heated in a distillation flask with 100 ml. of hydrochloric acid of 1.06 sp. gr. and successive portions of about 30 ml. distilled into a receiver, new portions of acid being added to the flask for each quantity distilled. The distillates are then tested for the presence of furfural, which in large amounts can usually be detected by its pleasant aromatic odor somewhat resembling that of bitter almond oil. The presence of very small amounts of furfural is best indicated by Schiff's reaction with aniline or xylidine acetate. Aniline acetate reagent is best prepared according to Tollens by mixing in a test tube equal volumes of aniline and water and then adding with constant shaking glacial acetic acid drop by drop until the milky solution becomes clear. Test paper is prepared by moistening strips of filter paper with the aniline acetate solution. Application of a drop of distillate containing furfural, even in minute traces, will cause the aniline acetate paper to turn a bright cherry red.

The presence of furfural in the distillate may also be indicated by first neutralizing the acid solution with sodium carbonate and then adding a solution of phenylhydrazine acetate and stirring. Furfural if present is precipitated as furfuralphenylhydrazone, $C_4H_3OCHN_2HC_6H_5$, which melts at 97° to 98° C.

A better precipitating agent for furfural than phenylhydrazine is phloroglucinol. A solution of this compound in hydrochloric acid when added to a distillate containing furfural will cause an immediate dark-

[92] *Landw. Vers.-Stat.*, 39, 425 (1891).

ening of the solution with final precipitation of furfuralphloroglucide, according to the equation:

$$\underset{\text{Furfural}}{C_5H_4O_2} + \underset{\text{Phloroglucinol}}{C_6H_6O_3} \longrightarrow \underset{\text{Furfuralphloroglucide}}{C_{11}H_8O_4} + \underset{\text{Water}}{H_2O}$$

Limitations of Furfural Reaction for Pentoses. While all carbohydrates containing a pentose group yield large amounts of furfural upon distillation with hydrochloric acid, it must also be borne in mind that other substances react similarly. All hexose carbohydrates such as starch, cellulose, sucrose, and glucose give small amounts of hydroxymethylfurfural upon distillation with hydrochloric acid but the yield is too small to interfere seriously with the test for pentoses. One other group of substances, however, is especially marked by its property of yielding furfural upon distillation with acids, and hence requires to be mentioned here, namely the hexuronic acids. Three representatives of this group have been found to occur in nature: glucuronic, galacturonic, and mannuronic acid. These acids are derived from the corresponding hexoses by the conversion of the primary alcohol group into a carboxyl group:

$$CH_2OH-(CHOH)_4-COH \rightarrow COOH-(CHOH)_4-COH$$

Distillation with acid splits off carbon dioxide:

$$COOH-(CHOH)_4-COH \longrightarrow CH_2OH-(CHOH)_3-COH + CO_2$$

and the pentose thus formed yields furfural, as shown on p. 703.

Glucuronic acid occurs in gum arabic, in certain saponins, in oxycellulose, and other plant and animal products. It is found in small quantities in normal urine, and in larger quantities after the ingestion of chloral, menthol, camphor, turpentine, acetanilide, alkaloids, and many other compounds. Under such conditions a combination takes place in the animal organism between the ingested compound and the glucuronic acid, the latter apparently being formed as an oxidation product of glycogen for the purpose of eliminating the toxic compounds enumerated above. The glucuronic acid derivative, which is excreted in the urine, may be mistaken for a pentose sugar if the chemist relies solely upon such tests as the furfural reaction and reduction of metallic salt solutions.

Galacturonic acid is the principal constituent of pectic acid and is also found in saponins, etc. Mannuronic acid has been obtained from various algae. All these substances give the furfural reaction, and it is necessary to apply special tests to distinguish the uronic acids from

pentoses. Such methods are described under the color reactions for sugar groups.

Methylfurfural Reaction for Methylpentose Groups. In the same way that all substances containing pentose groups yield furfural upon distillation with hydrochloric acid, those materials containing methylpentose groups yield methylfurfural. According to equation II on p. 703 the theoretical yield of methylfurfural is 67.07 per cent. In actual distillation experiments with the methylpentoses fucose and rhamnose, only from 35 to 40 per cent methylfurfural is obtained or 50 to 60 per cent of the theoretical amount.

In testing natural products for the presence of methylpentose groups, the material is distilled with hydrochloric acid of 1.06 sp. gr. in exactly the same manner as described for pentoses and the distillate is tested for methylfurfural. If no furfural is present in the distillate the presence of methylfurfural will be indicated by aniline acetate paper, which in this instance is colored yellow. If pentosans are also present in the plant material being examined, as they nearly always are, the presence of furfural in the distillate will color the aniline acetate paper red and completely mask the yellow color of the methylfurfural reaction. Other tests must, therefore, be employed to detect the presence of methylfurfural.

Maquenne[93] has devised a reaction by which 1 part methylfurfural can be detected in the presence of 9 parts furfural. A small amount of the solution to be tested is added to a mixture containing 3 volumes 95 per cent alcohol and 1 volume concentrated sulfuric acid, and the whole is gently warmed. The development of a bright grass-green color throughout the body of the solution indicates the presence of methylfurfural.

Spectral reactions for methylfurfural will be described in a succeeding section.

Reactions for Tetrose and Triose Groups. Except on the hexoses, pentoses, and methylpentoses, but few experiments have been made concerning the reactions of sugar groups with hydrochloric acid.

Experiments of Tollens and Ellett[94] show that *l*-erythrose is decomposed upon heating with hydrochloric acid into lactic acid. The reaction may proceed as follows:

$$\underset{\text{Tetrose}}{C_4H_8O_4} \longrightarrow \underset{\text{Lactic acid}}{C_3H_6O_3} + \underset{\text{Formaldehyde}}{CH_2O}$$

Tollens and Ellett suggest that the above may be a general reaction

[93] *Compt. rend.*, **109**, 573 (1889).

[94] *Ber.*, **38**, 499 (1905).

for tetrose groups, just as levulinic acid is formed from hexoses, furfural from pentoses, and methylfurfural from methylpentoses, but further investigations must be made upon the tetroses before any results from the above observations can be applied to sugar analysis.

The formation of considerable methylglyoxal, $CH_3—CO—COH$, by heating dihydroxyacetone, $C_3H_6O_3$, with 20 per cent sulfuric acid has been observed by Pinkus.[95] The same reaction is also given by the other triose, glyceric aldehyde.[96] Other carbohydrates do not interfere. The methylglyoxal is best identified as the *p*-nitrophenylosazone which crystallizes in red needles, melting at 302–304° C. with decomposition.

III. COLOR AND SPECTRAL REACTIONS WITH PHENOLS AS A MEANS OF IDENTIFYING SUGARS

A study of the color reactions and absorption spectra which solutions of different sugars give with various phenols as α-naphthol, orcinol, resorcinol, naphthoresorcinol, and phloroglucinol, in the presence of concentrated sulfuric or hydrochloric acids, offers frequently a most rapid as well as most reliable method for detecting sugar groups. These reactions are based on the formation of furfural or its derivatives by the effect of the acid, and the coupling of these products with phenols, giving rise to intensely colored compounds.[97]

Color Reactions of Ketoses. Reference has already been made (p. 658) to the greater ease with which solutions of ketoses show coloration phenomena in contact with concentrated sulfuric acid. The same fact has been noted with the colorations produced with sugars and α-naphthol and sulfuric acid, and this has been utilized as one means of detecting the presence of ketose sugars in mixtures.

α-Naphthol Test. Pinoff[98] has modified the α-naphthol test for sugars by using a mixture of 750 ml. 96 per cent alcohol and 200 ml. concentrated sulfuric acid as the condensing agent. By treating in a test tube 0.05 g. of sugar with 10 ml. of the alcohol-acid mixture and 0.2 ml. of alcoholic α-naphthol (5 g. α-naphthol dissolved in 100 ml. 96 per cent alcohol) and heating in boiling water, Pinoff obtained red colorations which with sugars containing ketone groups appeared almost immediately; with the aldose sugars 20 minutes or more elapsed before coloration developed. The following Table XCIV for 11 different sugars by Pinoff gives the time of heating before coloration, the number

[95] *Ber.*, 31, 31 (1898).
[96] Neuberg, Lewite, and Schwenk, *Biochem. Z.*, 83, 244 (1917).
[97] Middendorp, *Z. Ver. deut. Zucker-Ind.*, 74, 338 (1924).
[98] *Ber.*, 38, 3314 (1905).

of absorption bands shown by the solution before the spectroscope, and the position of the bands with reference to the wavelength of the light absorbed.

TABLE XCIV

ABSORPTION SPECTRA OF SUGARS WITH α-NAPHTHOL AND SULFURIC ACID IN ALCOHOL

Sugar	Time for Development of Color	Number of Absorption Bands	Wavelength in mμ and Position of Bands
	minutes		
Arabinose	20	..	.....
Rhamnose	20	1	562.5 (in yellow)
Glucose	35	1	532.5 (between yellow and green)
Mannose	31	1	532.5 (between yellow and green)
Galactose	31	1	532.5 (between yellow and green)
Fructose	1	2	573.6 (in yellow), 508.8 (in green)
Sorbose	1	2	573.6 (in yellow), 508.8 (in green)
Sucrose	1	2	573.6 (in yellow), 508.8 (in green)
Lactose	31	1	532.5 (between yellow and green)
Maltose	31	1	532.5 (between yellow and green)
Raffinose	1	2	573.6 (in yellow), 508.8 (in green)

It will be noted that for the ketose sugars fructose and sorbose and for the di- and tri-saccharides sucrose and raffinose, which give the ketose sugar fructose upon hydrolysis, a red coloration is obtained in 1 minute, whereas for the other sugars 20 to 35 minutes must elapse before coloration. Van der Haar[99] recommends heating for 3 minutes; as little as 3 mg. of fructose can thus be detected in the presence of 300 mg. of aldoses. By diluting the 10 ml. of sulfuric acid-alcohol mixture with 10 ml. of 96 per cent alcohol before making the test, Pinoff obtained no coloration sufficient to show absorption bands with any of the aldose sugars. For the ketose sugars he obtained the following results:

Sugar	Time for Development of Color	Number of Bands	Wavelength in mμ and Position of Bands
	minutes		
Fructose	13	1	508.8 (in green)
Sorbose	30	1	508.8 (in green)
Sucrose	15	1	508.8 (in green)
Raffinose	19	1	508.8 (in green)

While diluting the acid-alcohol mixture has practically eliminated

[99] "Anleitung zum Nachweis, zur Trenning und Bestimmung der Monosaccharide," p. 92, 1920.

the aldoses from the reaction, it has also materially lessened the sensibility of the test for the ketoses.

Foulger[100] has developed a technique by which aldoses and ketoses may be distinguished primarily by the location of the absorption bands. One milliliter of the sugar solution is mixed in a small flask with 10 ml. of 75 per cent sulfuric acid. One-tenth milliliter of a 3 per cent solution of α-naphthol is added, the well-shaken mixture placed in a water bath at 45° C. for 20 minutes, cooled, and examined in a spectroscope or spectrophotometer. The absorption band for aldoses lies within 480 and 510 mμ; that for ketoses, within 550 and 580 mμ. The test has been used for detecting ketoses in blood, urine, and spinal fluid.

The difference in the reaction rates of aldoses and ketoses has been utilized by Romani[101] for detecting added sucrose in milk. One drop of milk is mixed with 2 drops of 20 per cent α-naphthol solution and 3 ml. concentrated hydrochloric acid. The mixture is boiled for 3 to 4 seconds, cooled, and shaken with chloroform. With pure milk the chloroform remains colorless, but added sucrose gives a yellow to red color, depending on the quantity of the sucrose.

Resorcinol Test. The most convenient color test for distinguishing ketose from aldose sugars is the color reaction with resorcinol and hydrochloric acid, generally known as Seliwanoff's test.[102] The test was originally regarded as peculiar to fructose, but later experiments have shown that it is given by sorbose, tagatose, the ketopentoses, and all other sugars having a keto group.

The reaction is carried out, according to van der Haar's[103] modification, by mixing about 50 mg. of the sample with 10 ml. of *N* hydrochloric acid and about 10 mg. of resorcinol, and heating in a boiling-water bath for as long as 15 minutes. If a solution is to be tested, the strength of the acid is adjusted so that the final solution is of normal acid concentration. If fructose or other ketose is present a fiery eosin-red color will develop.

If the acid solution is made alkaline with soda and then shaken with amyl alcohol, the red coloring matter is dissolved with a greenish fluorescence. If a few drops of absolute alcohol are added now the color becomes a beautiful rose red.

If the red-colored solutions obtained by Seliwanoff's reaction are

[100] *J. Biol. Chem.*, 92, 345 (1931).
[101] *Ann. chim. applicata*, 21, 535 (1931).
[102] *Ber.*, 20, 181 (1887).
[103] "Anleitung zum Nachweis, zur Bestimmung und Trennung der Monosaccharide," p. 95, 1920.

examined before the spectroscope a distinct absorption band will be noted in the blue near the F line (see Fig. 272).

It is important in making the test with resorcinol that an excess of hydrochloric acid be avoided. If too much strong acid is present, glucose and other aldoses will also react with resorcinol and form pink-colored solutions which, though lacking the intensity of color obtained with the ketoses, may nevertheless lead to erroneous conclusions. The resorcinol reaction obtained with aldohexoses is due to the splitting off of small quantities of hydroxymethylfurfural, about one-twentieth of that obtained from ketoses under the same conditions.

Pinoff[104] has modified the resorcinol test for ketoses by using the alcohol-sulfuric acid mixture previously described as the condensing agent. In making the test 0.05 g. of sugar was treated in a test tube with 5 ml. of the alcohol-sulfuric acid reagent, 5 ml. alcohol, and 0.2 ml. of a 5 per cent resorcinol solution and the mixture placed in boiling water. Table XCV by Pinoff, for eleven different sugars, shows the length of time required for development of color, the number of absorption bands, and the position of the bands with reference to wave-length of light absorbed.

TABLE XCV

ABSORPTION SPECTRA OF SUGARS WITH RESORCINOL AND SULFURIC ACID IN ALCOHOL

Sugar	Time for Development of Color	Number of Absorption Bands	Wavelengths in mμ and Position of Bands
	minutes		
Arabinose	35	..	.....
Rhamnose	35	..	.....
Glucose	32	1	487.5 (in blue)
Mannose	35	..	.....
Galactose	35	..	.....
Fructose	1	1	487.5 (in blue)
Sorbose	1	1	487.5 (in blue)
Sucrose	1	1	487.5 (in blue)
Lactose	32	1	487.5 (in blue)
Maltose	32	1	487.5 (in blue)
Raffinose	1	1	487.5 (in blue)

Ofner[105] recommends the following standardized procedure for the Seliwanoff test. The reagent is prepared by mixing 60 ml. concentrated hydrochloric acid with 30 ml. water in a 100-ml. flask, mixing, cooling, diluting to the mark with water, and dissolving 0.5 g. pure resorcinol

[104] *Ber.*, **38**, 3314 (1905).

[105] *Chem. Ztg.*, **53**, 682 (1929).

in this dilute acid. Five milliliters of the sugar solution to be tested, containing not more than 3 per cent sugar, is mixed with 5 ml. of the resorcinol reagent, a little powdered pumice is added, the mixture boiled for exactly 20 seconds, cooled quickly, and examined after 2 minutes. Under these conditions 1 mg. of fructose or 2 mg. of sucrose produces a perceptible red color.

To detect added sucrose in milk, according to Castiglioni,[106] 1 ml. of the sample is mixed with 2 ml. of a 20 per cent alcoholic solution of resorcinol and 10 ml. concentrated hydrochloric acid, and the mixture is placed in a water bath heated to 50° C. If no red color develops in 5 minutes, sucrose is absent. One-fifth of 1 per cent of sucrose gives a positive test in 45 seconds. The procedure may be made quantitative by comparison with standards treated in the same way.

White and Green[107] have applied the Seliwanoff reaction to urine, for the detection of fructose, in the following manner. Add to the urine sample an equal quantity of concentrated hydrochloric acid, heat just to boiling, let stand for 2 minutes, shake with a little decolorizing carbon, heat again to boiling, let stand for 2 minutes, and filter. Eight drops of the filtrate is boiled for 30 seconds with 5 ml. of a solution of resorcinol in 12 per cent hydrochloric acid. By this method 0.3 per cent of fructose can be detected in the presence of 3 per cent of glucose.

The resorcinol test has been greatly improved and made more specific by Weehuizen.[108] The solid sugar, or a solution evaporated to a heavy sirup, is mixed with 3 to 4 ml. of absolute alcohol saturated in the cold with dry hydrochloric acid gas, and 50 mg. of resorcinol. In the presence of ketoses a cherry-red color develops at room temperature within about 3 minutes. The disturbing side reactions which take place at high temperatures are thus avoided, and a positive test is often obtained when other ketose reactions give doubtful results.

Kruisheer[109] recommends first removing all aldoses present by oxidation with hypoiodite. Two milliliters of the sample, containing not over 2 per cent sugar, is mixed with 0.5 ml. 4 *N* sodium hydroxide and 2 ml. 0.1 *N* iodine solution, and the mixture is allowed to stand for 5 minutes at room temperature. Then 4 ml. of 12 *N* hydrochloric acid and 4 ml. of 25 per cent copper sulfate solution are added, and the excess iodine is removed with sodium sulfite solution. Eight to ten milliliters of the clear supernatant liquid is pipetted off and subjected to the Seliwanoff test.

[106] *Ann. chim. applicata*, **22**, 641 (1932).
[107] *Trans. Roy. Soc. Can.*, Sect. V, **26**, 145 (1932).
[108] *Pharm. Weekblad*, **55**, 831 (1918).
[109] *Rec. trav. chim.*, **51**, 273 (1932).

Resorcinol Test for Detecting Artificial Invert Sugar in Honey. The ready formation of hydroxymethylfurfural from ketohexoses is utilized in the Fiehe[110] test for detecting the addition of commercial invert sugar to natural honey. Commercial invert sugar is usually prepared by heating concentrated sucrose solutions with a small amount of tartaric, citric, or hydrochloric acid to 110 to 120° C., and then cooling rapidly. Under these conditions perceptible amounts of hydroxymethylfurfural are formed from the fructose obtained by inversion.

In making the test, the honey is rubbed up with ether in a mortar, or shaken with it in a test tube. The ether solution is then filtered into a small porcelain dish. After evaporation of the ether the residue is heated with a 1 per cent solution of resorcinol in concentrated hydrochloric acid. In the presence of commercial invert sugar a red color develops which soon changes to a reddish brown.

According to Nelson[111] the heavy honey does not readily mix with the ether, or if it does an emulsion is formed. In the first case the hydroxymethylfurfural is only incompletely extracted, and in the second the ether cannot be separated from the honey. For this reason the Association of Official Agricultural Chemists[112] has modified the test by shaking 10 ml. of a 50 per cent solution of the honey in a test tube with 5 ml. of ether. After the tube has been allowed to stand until the ether solution is clear, 2 ml. is withdrawn into a small test tube, and a large drop of the resorcinol solution is added. If a cherry-red color appears at once the presence of commercial invert sugar is indicated.

In interpreting the result of the test it must be borne in mind that hydroxymethylfurfural is formed also when natural products containing fructose, such as honey, are cooked or heated to 160° F. or stored for long periods of time, as is sometimes done in processing honey. On the other hand, a negative result does not prove the absence of commercial invert sugar because small quantities of hydroxymethylfurfural may escape detection.

Another color test for detecting hydroxymethylfurfural, with aniline salts, is described on p. 720.

Naphthoresorcinol Test. Tollens and Rorive[113] have employed, in place of resorcinol, naphthoresorcinol or 1,3 dihydroxynaphthalene. The ketose sugars fructose and sorbose and the di- and trisaccharides sucrose and raffinose show upon heating with a little naphthoresorcinol

110 *Z. Untersuch. Nahr. u. Genussm.*, **16**, 75 (1908).

111 *J. Assoc. Official Agr. Chem.*, **12**, 323 (1929).

112 "Methods of Analysis, A. O. A. C.," 5th ed., p. 511, 1940. See also Bryan, *U. S. Bur. Chem. Bull.* **154**, p. 15, 1912.

113 *Ber.*, **41**, 1783 (1908).

in the presence of hydrochloric acid (1 vol. acid 1.19 sp. gr. and 1 vol. water) beautiful red-colored solutions which show a weak absorption band in the green. The sensibility of this test is about the same as that obtained in Seliwanoff's reaction, but the color has more of a violet tinge than the fiery red obtained with resorcinol. The red-colored solutions obtained with naphthoresorcinol soon become turbid with the formation of a deposit. If this is filtered off and dissolved in alcohol a yellowish brown solution with green fluorescence is obtained which shows a weak absorption band in the green.

The naphthoresorcinol test is little used for detecting ketoses in the presence of aldoses because this phenol gives very similar reactions with pentoses and hexuronic acids.

Color Reactions of Pentoses and Hexuronic Acids. The pentoses are distinguished above all other sugar groups for the depth and variety of the color reactions obtained with different polyphenols in the presence of concentrated hydrochloric acid. Phloroglucinol, orcinol, and naphthoresorcinol are the three compounds most used for this purpose, and the reactions for each of these will be described in the order named. The same reactions are given also by the hexuronic acids because they are converted into pentoses under the conditions of the test. Special methods must therefore be resorted to for the detection of these acids in the presence of sugars.

Phloroglucinol Test. Ihl[114] discovered that solutions of the pentose sugars, or of hydrolytic products derived from substances containing pentosans, gave, upon heating with an equal volume of concentrated hydrochloric acid and a little phloroglucinol, a beautiful violet-red color. The colored solution thus obtained when viewed before the spectroscope was found by Tollens and Allen[115] to show a sharp black absorption band in the yellow of the spectrum between the D and E lines.

The violet-red solution obtained in the phloroglucinol reaction for pentoses soon becomes turbid with deposition of a dark-colored precipitate. If the turbid solution is allowed to stand 3 to 5 minutes, then cooled and filtered, and the precipitate washed with cold water on a small rapid filter and then dissolved in 95 per cent alcohol, a permanent red solution is obtained which is perfectly adapted to the study of absorption spectra. If the color is too deep it can be reduced by careful dilution with 96 per cent alcohol (Tollens's " absatz " method). The coloring matter may also be extracted with pure amyl alcohol, and the solution examined with the spectroscope.

[114] *Chem. Ztg.*, 9, 231 (1885).
[115] *Ann.*, 260, 289 (1890).

The same color reaction is also given by hexuronic acids, but methylpentoses and oxycellulose give negative results. If methylpentoses are present in addition to pentoses the color produced is more reddish.

Orcinol Test. If the reaction for the pentoses just described is carried out with orcinol in place of phloroglucinol a violet-blue coloration is obtained. The solution, however, becomes rapidly turbid with deposition of a bluish-colored flaky precipitate. If this is filtered off and dissolved in alcohol by Tollens's " absatz " method a blue-colored solution is obtained which shows before the spectroscope a very sharp dark band almost exactly over the D line of the spectrum. The same reaction is also obtained with glucuronic acid.

Bial[116] has made the orcinol reaction more sensitive by carrying out the test in the presence of a little ferric chloride. In this manner it is found possible to distinguish between pentoses and glucuronic acid.

Bial's orcinol reagent is prepared by dissolving 1 g. orcinol in 500 ml. hydrochloric acid of 1.15 sp. gr. (30 per cent) to which 20 drops of an officinal solution of ferric chloride (liquor ferri sesquichloridi) is added.

In making the test 4 to 5 ml. of the reagent is heated in a test tube to boiling; the solution is removed from the flame and a few drops (never over 1 ml.) of the solution to be tested added. If pentoses are present a vivid green color will develop almost immediately; the reaction is not given under the above conditions with glucuronic acid.

Bial's test has been studied and generally confirmed by Sachs[117] and also by Tollens and Lefèvre.[118] The last-named authorities found that a dilute solution of glucuronic acid produced no perceptible coloration under the conditions prescribed by Bial, but that if the solution was heated for any length of time a green color speedily developed. The cause of the retardation is explained by the slower decomposition of glucuronic acid by hydrochloric acid as compared with the pentoses; such a difference in the rate of decomposition is also noted between the pentose sugars themselves, xylose, for example, giving a coloration with Bial's reagent in a shorter time than arabinose. Other hexuronic acids give a reaction similar to that of glucuronic acid.

The green solution obtained by Bial's reaction shows before the spectroscope a dark absorption band in the red between the lines B and C and a second, weaker band in the yellow covering the position of the D line of the spectrum. Methylpentoses produce only the darker band, not the second one.

[116] *Chem. Zentr.*, 1902, II, 295; 1903, II, 1021.
[117] *Biochem. Z.*, 1, 384 (1906).
[118] *Ber.*, 40, 4520 (1907).

Another variant of the orcinol test has been introduced by Neumann[119] and has been found very useful by van der Haar.[120] In this modification 20 to 30 mg. of the sample is dissolved in 10 drops of water, 5 ml. of glacial acetic acid and 5 drops of a 5 per cent solution of orcinol in alcohol are added, and the mixture is heated to boiling. Ten drops of sulfuric acid is added, and the mixture is examined with the spectroscope in a layer 2 and 4 cm. thick. Pentoses and hexuronic acids give a blue to green solution, with two absorption bands in the same position as in Bial's test. Methylpentoses and hexoses give a yellow to brown color and no absorption bands. In the presence of large quantities of these other sugars the reaction for pentoses is less sensitive.

Napthoresorcinol Test for Pentoses. Tollens and Rorive[121] have found that when solutions of different sugars are heated with a little naphthoresorcinol in the presence of an equal volume of concentrated hydrochloric acid (1.19 sp. gr.) characteristically colored solutions and deposits are formed.

With the pentoses arabinose and xylose a red color develops on heating followed by a bluish turbidity. The deposit dissolves in alcohol to a reddish brown solution with beautiful green fluorescence, showing a weakly defined absorption band in the green.

Although naphthoresorcinol gives color reactions with hexoses and pentoses it has been found most useful for the detection of hexuronic acids.

Naphthoresorcinol Test for Hexuronic Acids. When the naphthoresorcinol test just mentioned is applied to glucuronic acid or its derivatives, a bluish turbid solution is obtained, with a blue deposit. The alcoholic solution of the latter is a beautiful blue, only slightly fluorescent, and shows a dark absorption band in the yellow covering the D line of the spectrum. Galacturonic acid gives the same reaction.

The naphthoresorcinol test for hexuronic acids has been improved by Tollens[122] in the following way. The deposit of coloring matter is treated with ether instead of alcohol; if glucuronic acid is present the ether is colored a violet blue and shows before the spectroscope an absorption band in the yellow, its center lying a little to the right of the D sodium line (i.e., toward the green).

The naphthoresorcinol deposits obtained with sugars (pentoses, hexoses, etc.) in the presence of hydrochloric acid are insoluble in ether

[119] *Berlin. klin. Wochsch.*, 41, 1073 (1904).

[120] "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide," p. 44, 1920.

[121] *Ber.*, 41, 1783 (1908).

[122] *Ber.*, 41, 1788 (1908).

and so do not appear in the reaction. The presence of sugar and also of foreign organic matter, as in urine, may change the color of the ether solution from the violet-blue characteristic of pure glucuronic acid to a violet, red, or reddish brown. The characteristic absorption band in the yellow part of the spectrum, however, will not be interfered with.

The naphthoresorcinol test as prescribed by Tollens is made as follows: 5 to 6 ml. of the solution (urine, etc.) to be tested is treated in a 16-mm.-wide test tube with ½ to 1 ml. of a 1 per cent solution of naphthoresorcinol in alcohol, and an equal volume of hydrochloric acid of 1.19 sp. gr. is added. The solution is carefully heated to boiling and then kept for 1 minute over a small flame. The dark-colored solution is set aside for 4 minutes and then cooled under a stream of cold water; an equal volume of ether is then added and the whole thoroughly shaken. After the acid solution has settled the ether layer will be colored blue or bluish violet to red, if glucuronic acid is present, and, if the tube is held before the spectroscope, will show the characteristic absorption band near the D line. If the ether does not separate readily a drop or two of alcohol will hasten the process. If the ether solution is too deeply colored for spectroscopic examination more ether is added until the color is reduced and the unabsorbed part of the spectrum made visible.

The naphthoresorcinol deposits of the pentoses and other sugars being insoluble in ether separate as a layer between the colored ether and the lower acid solution.

Van der Haar[123] observed that benzene, first recommended by Neuberg and Saneyoshi,[124] is better than ether for extracting the coloring matter obtained with hexuronic acids. In the case of pentoses, methylpentoses, or aldohexoses the benzene remains colorless, only fructose giving a yellow to brown solution. Hexuronic acids give the characteristic violet color, with the absorption band near the D line.

Neuberg and Kobel[125] have found that hexuronic acids may be detected in the presence of a large excess of pentoses or other sugars by using a more dilute acid than specified by Tollens. Three milliliters of 2 *N* hydrochloric acid is added to an equal volume of solution containing 0.1 per cent uronic acid and 0.1 g. naphthoresorcinol. The mixture is heated in a boiling-water bath for 5 minutes with constant shaking. In the presence of uronic acid a fine flocculent precipitate forms which can be extracted with ether, benzene, toluene, or chloroform.

[123] "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide," p. 55, 1920.

[124] *Biochem. Z.*, **36, 56 (1911).**

[125] *Biochem. Z.*, **243, 435 (1931).**

β-Naphthol Test. According to Thomas,[126] β-naphthol may be used for differentiating between pentoses and hexuronic acids. When an aqueous solution of a pentose is added, without mixing, to a 0.3 per cent solution of β-naphthol in concentrated sulfuric acid, a pure blue ring appears at the interface. Glucuronic acid under the same conditions produces a ring of crimson red. Hexoses and methylpentoses give a green ring which changes to an intense brown.

Color Reactions of Methylpentoses. The color reactions for detection of methylpentoses may be divided into two classes: (1) color reactions made upon the distillate obtained by distilling methylpentoses or methylpentosans with hydrochloric acid; (2) color reactions made directly upon these substances without distillation. The color reactions of the first class are in reality color reactions of methylfurfural to which reference has already been made. It remains, however, to describe some of the spectral reactions of methylfurfural.

Spectral Reactions of Methylfurfural. Tollens and Widtsoe[127] have detected the presence of methylfurfural in the hydrochloric acid distillate from various plant materials by mixing a few milliliters of the solution with an equal volume of concentrated hydrochloric acid and gently warming. If the solution is colored yellow methylfurfural is present. The yellow solution viewed before the spectroscope will show a dark absorption band between the green and blue of the spectrum near the F line. If much methylfurfural is present the band will gradually darken and broaden, the increase in width extending toward the violet and leaving the green unaffected. With considerable methylfurfural the violet end of the spectrum is completely extinguished, the green, however, always remaining clear and transparent. Furfural does not give this reaction although it may affect the delicacy of the test if present in large amount. The reaction, however, will indicate 1 part of methylfurfural in the presence of 64 parts furfural (1/32 drop methylfurfural in the presence of 2 drops furfural in 10 ml. of hydrochloric acid). By this test Tollens and Widtsoe were able to detect methylpentosans in different gums, seaweed, leaves of different kinds of trees, and a large variety of other plant substances.

Tollens and Oshima[128] have rendered the spectral reaction for methylfurfural more sensitive by carrying out the test in the presence of phloroglucinol; 5 ml. of the hydrochloric acid distillate is treated with 5 ml. of concentrated hydrochloric acid and a few milliliters of a solution of phloroglucinol (in hydrochloric acid of 1.06 sp. gr.) added. After

[126] *Bull. soc. chim. biol.*, 7, 102 (1925).
[127] *Ber.*, 33, 146 (1900).
[128] *Ber.*, 34, 1425 (1901).

5 minutes the solution is filtered from the greenish black precipitate of furfural phloroglucide; if the filtrate is colored yellow or reddish yellow methylfurfural is present. Before the spectroscope the solution gives a dark absorption band in the blue. On long standing the solution deposits a red precipitate of methylfurfural phloroglucide which is readily distinguished from the dark green furfural compound. Absorption spectra of methylfurfural are shown in Fig. 272.

The vivid color reaction of pentoses with phloroglucinol and hydrochloric acid is not obtained with the methylpentoses. The reaction of methylpentoses with orcinol has been mentioned under pentoses (p. 716). Methylpentoses were found by Tollens and Rorive to give a color test with naphthoresorcinol also, but this reagent is of greater value for the detection of hexuronic acids (p. 717).

A few characteristic absorption spectra, useful in testing for sugars, are shown in Fig. 272.

IV. MISCELLANEOUS GROUP AND SPECIAL REACTIONS

In addition to the group reactions described in the preceding sections, various others have been found valuable for identifying sugar groups or individual sugars and sugar derivatives.

Reactions with Aromatic Amines and Other Organic Nitrogen Compounds. Furfural and its derivatives which form the basis of the color reactions with phenols may be detected also by means of aromatic amines and other organic nitrogen compounds. A few examples of such reactions will suffice.

Aniline Test.[129] This test, which has already been mentioned on p. 714, may also be obtained without previous distillation with acid. Two milliliters of glacial acetic acid and 5 drops of redistilled aniline are added to 2 ml. of sugar solution, the mixture is boiled and allowed to stand for 2 minutes, and the color is extracted with 2 ml. of chloroform. Pentoses give a characteristic bright red color with an absorption band in the blue, methylpentoses a yellow color without an absorption band, fructose pale yellow, glucose or galactose green.

Browne's Aniline Acetate Test for Detecting Artificial Invert Sugar.[130] This is a more rapid but less sensitive reaction than the one with resorcinol described on p. 714. The reagent should be freshly prepared before use by shaking up 5 ml. of chemically pure aniline with 5 ml. of water and adding sufficient glacial acetic acid (2 ml.) to just clear the emulsion. In making the test 5 ml. of a concentrated solution of the honey, etc., is treated in a test tube with 1 to 2 ml. of the aniline reagent.

[129] White and Green, *Trans. Roy. Soc. Can.*, Sect. V, **26, 145 (1932).**

[130] *U. S. Bur. Chem. Bull.* **110, p. 68.**

The latter is allowed to flow down the walls of the tube so as to form a layer upon the surface of the solution underneath. If a red ring forms

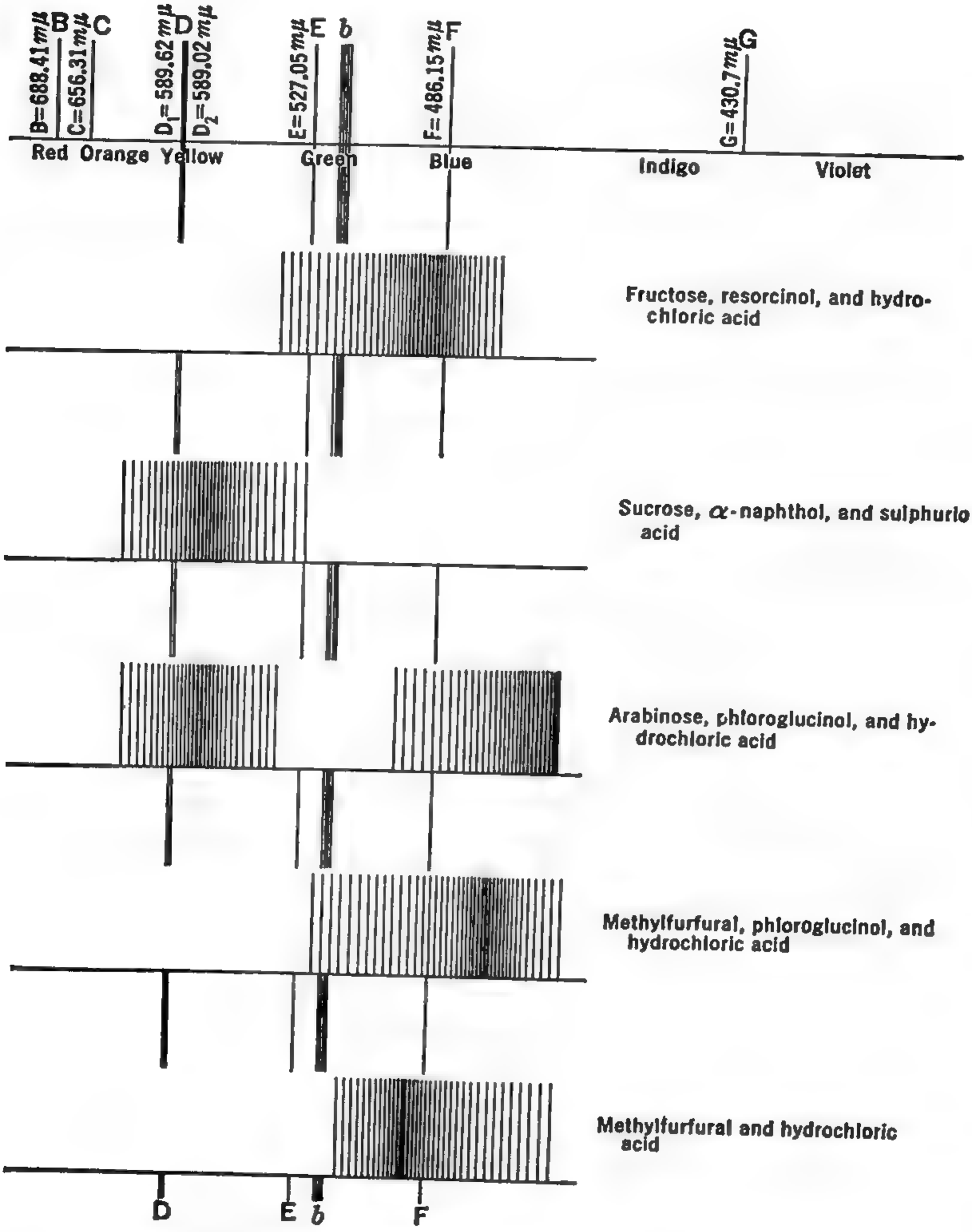


FIG. 272. Absorption spectra given by different sugars and by methylfurfural.

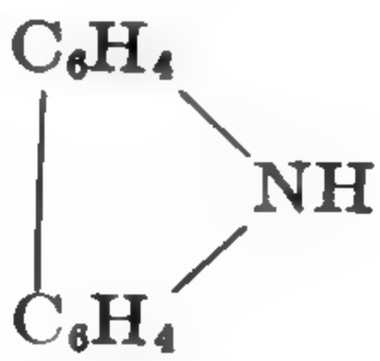
below the aniline solution, when the tube is gently agitated, hydroxymethylfurfural is present.

Feder's Aniline Chloride Test for Artificial Honey.[131] The reagent is prepared by dissolving 100 ml. of pure aniline with 30 ml. of 25 per cent hydrochloric acid. Five grams of honey is transferred to a porcelain dish and 2.5 ml. of the freshly prepared aniline reagent is added with stirring. In the presence of commercial invert sugar an orange-red color is formed immediately which changes to dark red upon standing. This method is used by the Association of Official Agricultural Chemists[132] as an alternative to the resorcinol test (p. 714).

Benzidine Test. This very sensitive test, described by Tauber,[133] is carried out with a solution of 1 g. benzidine, $H_2N—C_6H_4—C_6H_4—NH_2$, in 25 ml. of glacial acetic acid. One drop of sugar solution is mixed with 0.5 ml. of the reagent and heated to boiling. As little as 0.05 mg. of pentose or 0.2 mg. of glucuronic acid produces a cherry-red color which is quite permanent. Hexoses give a yellow to brown color. The combinations of glucuronic acid with phenolic compounds, occurring in urine, do not give the test, and pentoses may thus be detected in urine.

Diphenylamine Test. This reaction was discovered by Ihl.[134] The following procedure is due to Dische.[135] One volume of a 10 per cent alcoholic solution of diphenylamine, $(C_6H_5)_2NH$, is mixed with 4 volumes of glacial acetic acid and 5 volumes of concentrated hydrochloric acid. One volume of the solution to be tested and 2 volumes of the reagent are heated for 30 minutes on a water bath. Hexoses give a blue coloration, pentoses brown; ketoses react more rapidly than aldoses.

Carbazole Test.[136] Carbazole is dibenzopyrrole, of the formula



The test is made by using 1 ml. of sugar solution, adding 2 ml. of concentrated sulfuric acid, mixing and cooling, adding 0.1 ml. of a 0.5 per cent solution of carbazole in alcohol, mixing and cooling again, and then heating in a boiling-water bath for 10 minutes. A positive color test is obtained with solutions containing as little as 0.001 per cent of various sugars. Hexoses and pentoses give a violet or red color, glucu-

[131] *Z. Untersuch. Nahr. u. Genussm.* **22**, 412 (1911).
[132] "Methods of Analysis, A. O. A. C.," 5th ed., p. 511, 1940.
[133] *Proc. Soc. Exptl. Biol. Med.*, 37, 600 (1937); **38**, 171 (1938).
[134] *Chem. Ztg.*, **9**, 451 (1885).
[135] *Mikrochem.*, **7**, 33 (1929).
[136] Hepburn and Lazarchick, *Am. J. Pharm.*, **102**, 560 (1930).

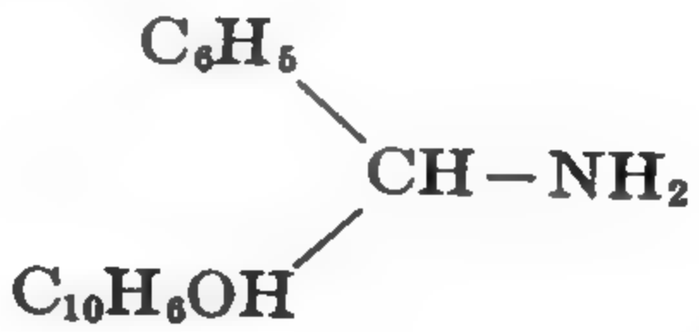
ronic acid the same but fainter, and trioses a blue color. Polysaccharides, glucosides, and similar derivatives also give this reaction.

Test for Ketohexoses with Thiobarbituric Acid. The fact that ketohexoses yield hydroxymethylfurfural more readily than the corresponding aldoses has been utilized by Plaisance[137] for the detection of the former in presence of the latter. The substance to be tested is brought to boiling in a test tube with 12 per cent hydrochloric acid. The mixture is cooled, and a few drops of a solution of thiobarbituric acid in 12 per cent hydrochloric acid are added. In the presence of ketohexoses, or of carbohydrates giving ketohexoses upon hydrolysis, an orange-colored precipitate forms on standing. Aldohexoses give a yellow color but no precipitate. Barbituric acid does not give an insoluble compound with hydroxymethylfurfural.

Jordan and Pryde's Test for Ketohexoses.[138] If 5 to 10 mg. of the sample is heated for ½ hour at 40° C. with a solution of 10 mg. of pure skatole in 10 ml. of concentrated hydrochloric acid, fructose and other ketohexoses give an intense purple color similar to that of permanganate solutions. Aldoses give a similar color upon heating for 15 minutes at 80° C. Ketoses can be detected by this test in the presence of a large excess of aldoses.

Fenton and Gostling's Test for Ketohexoses.[139] When ketohexoses are treated with hydrogen bromide in dry ether an intense purple color develops within less than an hour. This reaction is due to the formation of 4-bromomethylfurfural.

Specific Tests for Aldoses. Betti[140] discovered that β-naphtholbenzylamine, of the formula

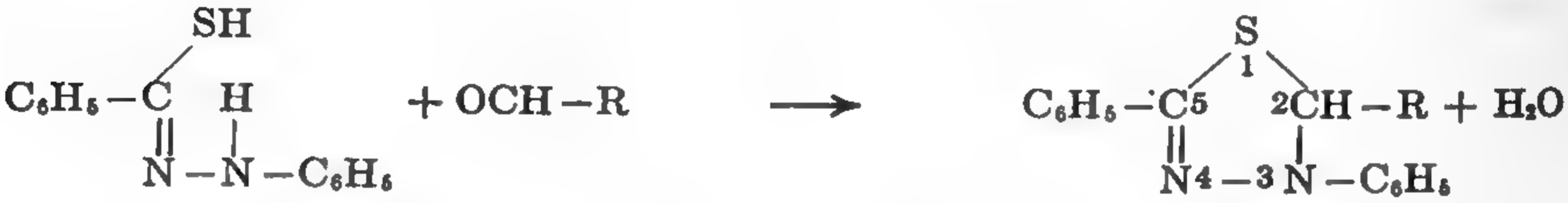


reacts, like other aromatic amines, with glucose, galactose, mannose, and rhamnose, yielding crystalline condensation products, but does not combine with fructose or sorbose. For example, glucose and fructose may be separated in the following manner. A solution of 0.9 g. of each of the two sugars in water and a little alcohol is added to a solution of 2.5 g. of the reagent in 95 per cent alcohol. The mixture is

[137] *J. Biol. Chem.*, **29**, 329 (1917).
[138] *Biochem. J.*, **32**, 279 (1938).
[139] *J. Chem. Soc.*, **73**, 554 (1897); see also Colin and Ruppol, *Bull. soc. chim. biol.*, **9**, 928 (1928).
[140] *Gazz. chim. ital.*, **48**, II, 288 (1912).

allowed to stand for 24 hours and then evaporated in a large dish. The residue is triturated with a little water, filtered, and washed with cold water. The solution contains the fructose. The residue on the filter is dried, the excess reagent extracted with benzene, and the insoluble portion recrystallized from alcohol. The condensation product of glucose melts at 192° C., and the glucose may be recovered from it by hydrolysis. The galactose compound melts at 206° C., that of mannose at 207 to 208° C., and the rhamnose compound at 192° C.

Another reaction by which aldoses may be distinguished from ketoses has been described by Wuyts.[141] The reagent used is α-phenyl-β-thiobenzoylhydrazide, and the reaction proceeds as follows:



giving a diphenyldihydrothiodiazole substituted at the 2 position by the aldose group. Two grams of the sugar sample and 2 g. of the reagent, both powdered, are mixed with 2 ml. of alcohol containing 5 per cent of hydrogen chloride, and the mixture is placed for a short time in a boiling-water bath. In the case of aldoses the mixture turns red, liquefies for a short time, and then solidifies. The reaction product is washed with water to remove uncombined sugar and is then recrystallized from ethyl or methyl alcohol. The glucose compound melts at 147 to 148° C., the galactose compound at 178 to 179° C., the mannose compound at 198° C. The arabinose compound must be crystallized from a mixture of pyridine and water; it melts at 222° C. Lactose reacts like the monosaccharides, but the product has not been obtained in crystalline form. When fructose is used instead of the aldoses the mixture with the reagent liquefies but does not solidify afterward.

Rosenthaler's Test for Methylpentoses.[142] Rosenthaler found that when methylpentoses are heated in a boiling-water bath with 10 ml. of 38 per cent hydrochloric acid and 1 to 2 ml. of acetone a violet color is produced which upon spectroscopic examination shows an absorption band in the region of the D line. According to van der Haar,[143] pentoses and hexoses give similar color reactions, but the absorption bands are located in other parts of the spectrum and disappear after a while. If the mixture is heated for 3 to 5 minutes, then cooled and allowed to

[141] *Compt. rend.*, **196**, 1678 (1933).

[142] *Z. anal. Chem.*, **48**, 165 (1909).

[143] "Anleitung zum Nachweis, zur Trennung und Bistimmung der Monosaccharide," p. 49, 1920.

stand, a permanent absorption band over the D line indicates the presence of a methylpentose. If hexoses are present in addition to methylpentoses and pentoses, the violet color assumes a brownish tinge, and in that case it is better to distil with hydrochloric acid according to the Tollens procedure and to test the distillate obtained.

Windaus's Reaction for Methylpentoses.[144] According to Windaus, acetaldehyde is obtained when methylpentoses are distilled with chromic acid (CrO_3) and glacial acetic acid. The acetaldehyde may be identified as the *p*-nitrophenylhydrazone, which melts at 128° C. Other sugars do not give this reaction. But when sulfuric acid is used instead of glacial acetic acid hexoses produce acetaldehyde also, by decomposition of the levulinic acid formed.

Tests for Individual Sugars. These may usually be identified by the hydrazones and osazones listed on pp. 684–688, by the acids formed upon oxidation with bromine or nitric acid, and by the alcohols obtained upon reduction with sodium amalgam. A few other characteristic tests follow:

Tests for Xylose. One of the best methods for detecting xylose in the presence of other sugars is *Bertrand's*[145] *reaction* by means of bromine and cadmium carbonate. The bromine oxidizes the xylose to xylonic acid according to the following reaction:

$$\begin{array}{l} \mathrm{CHO} \\ | \\ (\mathrm{CHOH})_3 \\ | \\ \mathrm{CH_2OH} \end{array} + \mathrm{H_2O} + \mathrm{Br_2} \longrightarrow \begin{array}{l} \mathrm{COOH} \\ | \\ (\mathrm{CHOH})_3 \\ | \\ \mathrm{CH_2OH} \end{array} + \quad 2\,\mathrm{HBr}$$

Xylose — Xylonic acid — Hydrobromic acid

The xylonic and hydrobromic acids react with the cadmium carbonate forming cadmium xylonate and bromide, the solution of which on evaporation deposits characteristic boat-shaped crystals of the double bromide and xylonate of cadmium $(C_5H_9O_6)_2Cd + CdBr_2 + 2\,H_2O$. The salt can be purified by recrystallizing and should show upon analysis 29.86 per cent Cd and 21.32 per cent Br.

Bertrand's reaction, according to Tollens and Widtsoe,[146] is carried out as follows. For each 0.2 g. sugar or double the amount of sirup to be tested, 1 ml. of water, 0.25 g. bromine (7 to 8 drops), and 0.5 g. cadmium carbonate are mixed together in a test tube with gentle warming and then, after corking loosely, set aside for 24 hours. The solution is then evaporated in a dish almost to dryness, taken up with a

[144] *Z. physiol. Chem.*, 100, 167 (1917).
[145] *Bull. soc. chim.*, [3], 5, 546 (1891).
[146] *Ber.*, 33, 132 (1900).

little water, filtered, and again evaporated almost to dryness. If xylose is present addition of a little alcohol will soon cause crystals of the double cadmium salt to deposit. Presence of impurities may delay the crystallization somewhat. Too much bromine must be avoided in making the test, and an excess of cadmium carbonate must always be present. The first crop of crystals frequently appears amorphous, but the characteristic boat-shaped needles are always obtained upon recrystallizing.

A second method which has been employed for the detection of *d*-xylose in impure mixtures is by means of the diformal[147] compound, which separates in crystalline form upon boiling xylose solutions with paraformaldehyde (trioxymethylene). *d*-Xylose diformal has the formula $C_5H_6O_5(CH_2)_2$; it consists of white crystals melting at 56° C.; it can be sublimed without decomposition, and it shows in methyl alcohol $[\alpha]_D = +25.7$.

Test for d-Arabinose. This sugar may be distinguished from *l*-arabinose by means of *l*-menthylhydrazine, which produces an insoluble hydrazone, while the hydrazone of *l*-arabinose is soluble (see p. 683).

Tests for Apiose. Unlike the pentose sugars, this sugar, having a branched carbon chain, does not give the furfural reaction. Reduction with hydrogen iodide and phosphorus yields isovalerianic acid. If a 0.3 per cent solution of β-naphthol in concentrated sulfuric acid is added, without mixing, to a solution of apiose, a green ring forms at the interface between the two solutions.

Saccharic Acid Test for Glucose. Glucose and the various substances which yield glucose upon hydrolysis are oxidized by strong nitric acid to saccharic acid, which is recognized by means of its acid potassium or silver salt. The test, according to Tollens and Gans,[148] is best carried out as follows:

Five grams of the material to be examined is treated in a porcelain dish with 30 ml. of nitric acid of 1.15 sp. gr., and the mixture is evaporated with constant stirring upon a boiling-water bath until evolution of red fumes has ceased and the resulting sirup has just begun to take on a permanent yellow color. The sirup is then taken up with a little water, heated over a flame, and powdered potassium carbonate added until a drop of the brownish-colored solution gives a blue reaction with red litmus paper. Glacial acetic acid is then added drop by drop until the mixture gives off a strong odor of acetic acid. If glucose was present in the original substance crystals of acid potassium saccharate will usually soon separate; if crystallization does not take place after a few

[147] Lobry de Bruyn and van Ekenstein, *Rec. trav. chim.*, **22**, 159 (1903).

[148] *Ber.*, **21**, 2149 (1888).

hours' standing, as may happen when only small amounts of glucose are present, the sirup should be concentrated further by gentle evaporation. After 24 hours' standing, the crystals which have formed are filtered off, or drained upon unglazed porcelain, and then recrystallized from the smallest possible amount of hot water. A third crystallization, using bone black, will usually eliminate the last traces of oxalic acid and other impurities and give a perfectly pure salt. The yield of acid potassium saccharate by this method is about 30 to 40 per cent of the original amount of glucose. The compound consists of shining rhombic crystals with characteristic trapezoidal faces, the appearance of which under the microscope is unmistakable. Acid potassium saccharate has the formula $COOH\ (CHOH)_4\ COOK$.

The acid potassium saccharate as above prepared can be further identified by conversion to the silver salt. For this purpose the acid potassium salt, after drying and weighing, is dissolved in a little water, to which ammonia is then added to the point of neutrality. The solution is then poured into a cold silver nitrate solution containing $AgNO_3$ to the amount of $1\frac{1}{2}$ the weight of the acid potassium salt taken. The precipitated saccharate of silver after standing a short time is filtered, washed free from silver nitrate, and then dried in a dark place over concentrated sulfuric acid. The silver saccharate has the formula $C_6H_8O_8Ag_2$ and upon ignition in a porcelain crucible should show 50.91 per cent silver.

In making the saccharic acid test for glucose it should be remembered that *d*-gluconic, *d*-glucuronic, and *d*-gulonic acids and *d*-gulose also give saccharic acid upon oxidation with nitric acid. This limitation, however, is a comparatively slight one, and the saccharic acid reaction upon the whole is one of the best tests for *d*-glucose in the presence of other sugars.

Identification of Glucuronic Acid. Glucuronic acid may be distinguished from glucose by the naphthoresorcinol reaction for uronic acids (p. 717), and may be further identified by the cinchonine salt which melts at 202° C. and has $[\alpha]_D = +139.9$.[149]

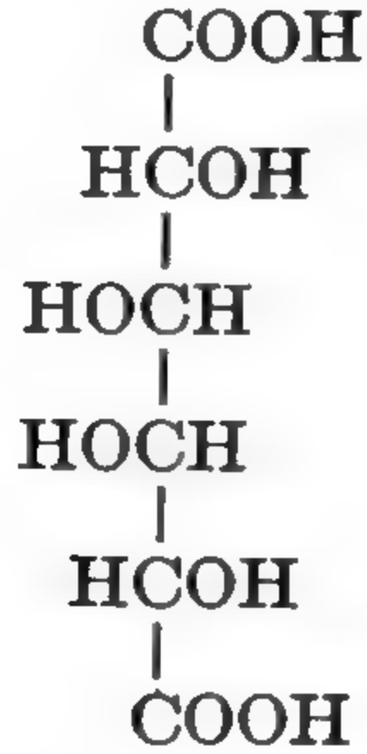
Glucose may also be removed by fermentation with yeast, and the unfermented residue tested for glucuronic acid by the saccharic acid or the naphthoresorcinol reaction. If a mixture of glucose and glucuronic acid in solution is evaporated with excess barium carbonate the glucuronic acid is converted into the barium salt which is insoluble in boiling 90 per cent alcohol whereas the glucose may be removed by repeating extraction with this solvent.

The combinations of glucuronic acid with phenolic compounds, oc-

[149] Neuberg, *Ber.*, 33, 3322 (1900).

curring in urine, are precipitated by basic lead acetate, but not by the neutral salt.

Mucic Acid Reaction for d-Galactose. The test most generally employed for detecting galactose either in the free or combined form is the production of mucic acid upon oxidation with nitric acid. Mucic acid has the formula



The reaction is carried out, according to Tollens and Kent,[150] by evaporating 5 g. of sample with 60 ml. of nitric acid of sp. gr. 1.15 on the water bath to one-third of the original volume, and allowing to stand for 24 hours. In the presence of galactose, crystals of mucic acid, consisting of minute granular rhombic prisms, are formed which may be separated by filtration through a Gooch crucible. If the galactose is accompanied by impurities the crystals are irregular in shape, and the mucic acid must be recrystallized by dissolving in dilute sodium hydroxide solution and acidifying again with hydrochloric acid. Or the mucic acid is converted into the thallium salt. It is dissolved on the microscope slide in a drop of dilute ammonia, a grain of thallium nitrate is added, and the slide is agitated until the thallium salt is dissolved. Upon standing, prismatic rods of thallium mucate separate out.[151]

Mucic acid melts at 213 to 214° C. and is almost insoluble in water (1 part in 300 parts of water). It is optically inactive and contains 34.27 per cent carbon, 4.8 per cent hydrogen.

It must be remembered that the mucic acid reaction is not specific for *d*-galactose but is given also by *l*-galactose, galactonic acid, galacturonic acid, lactose, dulcitol, and quercitol.

Identification of Galacturonic Acid. To distinguish galacturonic acid from galactose, the naphthoresorcinol test, the fermentation

[150] *Ann.*, 227, 222 (1885).

[151] Van der Haar, " Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide," p. 103, 1920.

method, or the barium carbonate procedure may be used, as described for glucuronic acid (p. 727). Galacturonic acid may also be identified by the following specific reaction, due to Ehrlich:[152] If basic lead acetate solution is added to a solution of galacturonic acid a white flocculent precipitate is formed which dissolves in an excess of the lead reagent. Upon boiling of the solution a brick-red precipitate is obtained. The cinchonine salt of galacturonic acid melts at 178° C.

Color Test for Mannose.[153] A 1 per cent solution of heroine in concentrated sulfuric acid gives a purple to violet color with mannose, while with other carbohydrates the color is amber, red, or brown. Codeine gives a similar reaction, which is not so characteristic, however.

Tests for d-Fructose.[158] If a fructose solution is heated with a dilute solution of cobaltous chloride, and then a little ammonium hydroxide is added, a violet or purple color develops. With other sugars green cobaltous hydroxide is obtained.

Kolthoff's[154] test is based on the oxidation of aldoses by hypoiodite and subsequent reduction of Fehling's solution. A 1 per cent solution of the sugar mixture is prepared, and 2 ml. of this is treated carefully with 4 ml. of 0.1 *N* iodine solution and 5 ml. of 2 *N* sodium hydroxide. The mixture is shaken, allowed to stand for 1 to 1½ hours, and then the excess iodine is removed with a few drops of *N* sodium thiosulfate solution. Then 4 ml. of Fehling's solution is added, and the mixture is heated not over 5 minutes in a boiling-water bath. If the sugar mixture contained 5 per cent of fructose, the cuprous oxide precipitate appears within 1 minute; with 2.5 per cent it is obtained in 2 minutes; and with 1 per cent, in 4 minutes. By this test fructose or another ketose can be detected in mixtures with glucose, lactose, maltose, or dextrin.

Detection of Traces of Invert Sugar in Cane Sugar. This test is carried out, according to von Morgenstern,[155] with a modified Barfoed solution (p. 648), in which the hydrolysis of the sucrose is prevented by the addition of sodium acetate and glycocoll. The reagent is prepared by adding 1 ml. of glacial acetic acid to 200 ml. of a solution of 1 part of crystallized neutral copper acetate in 15 parts of water. Twenty milliliters of this reagent is mixed with 2 g. sodium acetate and 0.1 to 0.15 g. of glycocoll, and 20 ml. of the 50 per cent sugar solution to be tested is added. The mixture is heated in a boiling-water bath for 5 minutes. A red precipitate formed during that time indicates the pres-

[152] *Ber.*, 65, 352 (1932).
[153] Dehn, Jackson, and Ballard, *Ind. Eng. Chem., Anal. Ed.*, 4, 414 (1932).
[154] *Chem. Weekblad*, 19, 1 (1922).
[155] *Centr. Zuckerind.*, 42, 824 (1934).

ence of invert sugar in the cane sugar. Fehling's solution may also be used for the same purpose, but the test must be carried out at a low temperature. If 10 ml. of a 50 per cent cane sugar solution and 10 ml. of Fehling's solution do not give a precipitate of cuprous oxide in 2 hours at 30° C., or in 20 minutes at 50° C., the sugar contains less than 0.001 per cent of invert sugar.

Tests for Lactose. Characteristic qualitative tests for lactose in the presence of other sugars are wanting. The formation of mucic acid upon oxidation with nitric acid is a valuable confirmatory test, although it must always be borne in mind that the same reaction is also given by galactose and its derivatives. Separation of lactosazone, after careful recrystallization and purification, offers a fairly reliable means of identification. If the phenylosazone is dissolved in hot water, oxidized with potassium permanganate and sulfuric acid, and the excess of permanganate removed with oxalic acid, the reaction product gives a positive test for formaldehyde.[156] If several reducing sugars are present, the mixture of osazones should be heated with boiling water and filtered; the osazones of lactose, maltose, and other disaccharides will be found in the filtrate, from which crystallization takes place upon cooling.

Neuberg and Saneyoshi[157] found that when lactosazone is boiled with dilute sulfuric acid it is split into glucosazone and galactose. The galactose can then be identified by the usual tests. Two hundred milligrams of lactosazone are boiled under reflux for 2 hours with 20 ml. of 2 per cent sulfuric acid. The reaction mixture is neutralized with an excess of barium carbonate, filtered, and the residue washed with a little hot water. The yellow filtrate containing the glucosazone and galactose is extracted repeatedly in a separatory funnel with ether until the ether layer is colorless. The aqueous layer is then boiled with a little bone black and filtered. The final filtrate is evaporated to a small volume and tested for galactose.

The osazone of melibiose, treated in the same manner, also gives glucosazone and galactose, but this is of little importance because lactose occurs only in animal products, melibiose only in plant materials.

In case of mixtures, the destruction of glucose, fructose, mannose, sucrose, maltose, and other fermentable sugars by means of yeasts which do not ferment lactose may be employed to advantage before making tests for lactose.

Tests for Maltose. What has been stated about the identification of lactose applies equally to that of maltose. The osazone reaction is one of the best means of identification, the greater solubility of

[156] Herzberg, *Biochem. Z.*, **119**, 81 (1921).
[157] *Z. Ver. deut. Zucker-Ind.*, **62**, 559 (1912).

maltosazone affording an easy means for its separation from the less-soluble osazones of other sugars; the influence of impurities in modifying the character of maltosazone, however, must always be considered. The test has been modified by Grimbert[158] by treating the impure maltosazone with a little cold aqueous 50 per cent acetone and filtering; the maltosazone separates from the filtrate in pure crystalline form.

Maltose may be identified by the method of Neuberg and Saneyoshi, described above for lactose, but in this case glucose is formed instead of galactose. The osazone of isomaltose also gives glucosazone and glucose, but isomaltose is readily distinguished from maltose by not being fermented by ordinary yeast.

Maltose, upon nitration with sulfuric and nitric acids, yields a characteristic octanitrate which melts at 163° to 164° C. and has $[\alpha]_D = +128.6$ in glacial acetic acid.[159]

The inability of certain yeasts, such as *Saccharomyces marxianus* or *S. exiguus*, to ferment maltose is another means of separation and identification which may be employed under certain conditions.

Tests for Melibiose. Reliable tests for melibiose in the presence of other sugars are lacking. The best method of procedure in case of mixtures is to remove glucose, fructose, and other sugars so far as possible by a pure culture of top yeast. The melibiose may then be precipitated as its phenylosazone; the latter is decomposed by benzaldehyde into melibiosone, which is hydrolyzed by emulsin into *d*-galactose and *d*-glucosone. Oxidation of melibiose or its osone with nitric acid yields mucic acid, in the same manner as with lactose.

Acetylation of melibiose produces an octa-acetate which melts at 177° C., and shows a specific rotation in chloroform $[\alpha]_D = +102.5$. Invertase does not affect melibiose, but the latter is hydrolyzed by the enzyme melibiase, occurring in bottom yeast.

Tests for Gentiobiose. As in the case of melibiose there are no characteristic tests for this sugar. It is not fermented by top yeast, and not hydrolyzed by invertase, but emulsin splits it into two molecules of *d*-glucose. The octa-acetate melts at 179 to 180° C. Its $[\alpha]_D$ in a mixture of equal parts of pyridine and alcohol = —72.2 initial, —44.4 final.

REACTIONS OF THE NON-REDUCING SUGARS

The comparatively small number of sugars which do not reduce Fehling's solution all belong to the higher di-, tri- and tetrasaccharides and include sucrose, trehalose, difructose anhydrides, raffinose, melezi-

[158] *J. pharm. chim.* [6], 17, 225 (1903).
[159] Will and Lenze, *Ber.*, 31, 84 (1898).

tose, gentianose, trifructosan, stachyose, and some other rare, incompletely studied higher saccharides. The soluble polysaccharides, such as dextrin, inulin, and glycogen, although not classified as sugars, are sometimes included for convenience in the group of non-reducing sugars.

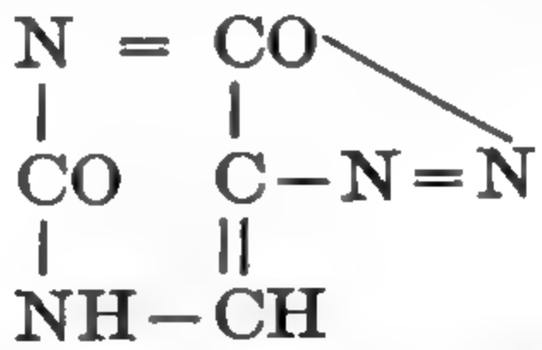
A free aldehyde or ketone group, to which the reducing sugars owe their peculiar reactivity in the formation of hydrazones, oximes, ureides, mercaptals, etc., is lacking in the non-reducing sugars, and the inability of the latter to reduce Fehling's solution, or to react with phenylhydrazine, dilute alkalies, hydroxylamine, etc., is thus explained.

The non-reducing sugars give many of the color and spectral reactions of the reducing sugars, sucrose and raffinose, for example, giving the reaction with α-naphthol and sulfuric acid, Seliwanoff's reaction with resorcinol and hydrochloric acid, etc. Some of these reactions have been described on pp. 709–715. But as previously explained these reactions are not given by the non-reducing sugar as such, but by the reducing sugars derived from it by the hydrolytic action of the acid used in making the test.

As in the case of most other sugars the only absolute means of identifying a non-reducing sugar is its separation in crystalline form and the determination of its physical and chemical properties. The most reliable confirmatory test is a carefully controlled hydrolysis with acid or enzymes, with measurements of the change in polarization and in copper-reducing power as a function of time, temperature, and other conditions. Methods involving this principle have been described under the inversion methods for determining sucrose and raffinose; other examples are given in the later chapters on quantitative methods.

Several special tests which may be used to detect the most commonly encountered non-reducing sugar, sucrose, are as follows:

Raybin's Test.[160] If 40 to 50 mg. sucrose, dissolved in a few milliliters of 0.05 *N* sodium hydroxide solution, is shaken at about 10° C. in a stoppered test tube with 7 to 10 mg. of diazouracil,



until the latter has dissolved, a blue-green color develops in a few minutes. In the presence of soluble magnesium salts a stable blue precipitate forms. This reaction is not given by glucose, fructose, or most other sugars, but by raffinose, gentianose, and stachyose all of

[160] *J. Am. Chem. Soc.*, **55**, 2603 (1933); **59**, 1402 (1937).

which are characterized by the fructofuranose-glucose linkage occurring in sucrose.

Pictet's Test for Sucrose. Pictet[161] observed that a mixture of cold, saturated solutions of sucrose and of copper sulfate in water, after standing for several hours, deposits microscopic needles of a double salt, $C_{12}H_{22}O_{11}$, $CuSO_4$, 4 H_2O. This reaction is not given by any of the other common pentoses, hexoses, di- and trisaccharides, or by the synthetic sucrose isomers. But in mixtures at least 10 per cent of sucrose must be present to produce a positive reaction.

Schlemmer's Test for Sucrose in the Presence of Invert Sugar.[162] This is based on the fact that reducing sugars are destroyed by boiling with milk of lime, and do not give the usual sugar reactions after this treatment. Twenty milliliters of the solution to be tested, containing not over 2 per cent of total sugar, is mixed in a test tube with 2 ml. of 10 per cent milk of lime, and the tube is placed in a boiling-water bath for 10 to 12 minutes. The tube is now centrifuged, the supernatant liquid pipetted off and tested with α-naphthol or thymol. If the color reaction cannot be readily seen because of the yellow tint of the treated sugar solution, a bichromate filter is used for the observation. This is easily accomplished by placing the test tube in which the α-naphthol reaction is carried out, in a vessel filled with a 0.25 to 0.5 per cent solution of potassium bichromate to match the color of the treated sugar solution. Under these conditions a positive test for sucrose may be obtained at a concentration of 0.001 per cent. Raffinose and reversion products of reducing sugars also give this test, also technical glucose which always contains reversion products. The test is therefore specific only in the absence of these other carbohydrates.

Rothenfusser's Test for Sucrose in Honey.[163] The difficulty caused by the yellow color of the sugar solution after treatment with milk of lime is avoided by using an oxidizing agent in addition to the alkaline earth oxide. Rothenfusser carries out the test as follows, to detect sucrose in honey. Dissolve 10 g. of honey in water to a volume of 100 ml. Take 10 ml. of this solution, add 50 ml. of acetone, shake for 1 minute, then add some diatomaceous earth, and shake for 1 minute longer. This precipitates dextrins which interfere with the reaction. Filter, add 10 ml. of water, and evaporate off the acetone. In the meantime dissolve 6 g. of barium hydroxide in 20 ml. of hot water, and add 25 ml. of 3 per cent hydrogen peroxide. The resulting reagent is mixed in a nickel dish with the honey filtrate, the dish placed on a boiling-

[161] *Helv. Chim. Acta*, 16, 144 (1933).
[162] *Z. Zuckerind. Čechoslovak. Rep.*, 51, 422 (1926/27).
[163] *Z. Untersuch. Nahr. u. Genussm.*, 24, 558 (1912).

water bath, and the mixture frequently stirred. When only a little yellow color remains, more hydrogen peroxide is added, and the heating continued until the liquid becomes colorless. Usually about 20 minutes' heating is sufficient. The mixture is then filtered, and 5 ml. of the filtrate is tested with 5 ml. of diphenylamine reagent prepared from 20 ml. of 10 per cent alcoholic diphenylamine solution, 60 ml. of glacial acetic acid, and 120 ml. of concentrated hydrochloric acid. The test tube is placed in a boiling-water bath for 7 to 8 minutes. If sucrose was originally present, the characteristic blue color develops. Rothenfusser did not investigate the behavior of raffinose or reversion products, and it is therefore doubtful whether the reaction is specific for sucrose.

SYSTEMATIC PROCEDURES FOR THE DETECTION OR IDENTIFICATION OF CARBOHYDRATES

In order to facilitate the identification of carbohydrates which the chemist may encounter, various schemes for a systematic procedure have been developed. The identification may be based on chemical reactions, on fermentation with yeast, fungi, or bacteria, or on the effects produced by enzymes. A few examples of such systems will be given.

Identification by Chemical Reactions. Dehn, Jackson, and Ballard[164] have compiled a table of chemical reagents used for the detection of carbohydrates, and another table showing the tests given with these reagents by a number of carbohydrates. These are reproduced in Tables XCVI and XCVII. The meaning of the figures and signs in Table XCVII is explained under " Remarks " in Table XCVI, and that of the abbreviations in a footnote below Table XCVII.

Some of the tests are characteristic for individual carbohydrates in the list, but they may also be given by closely related carbohydrates not included by Dehn, Jackson, and Ballard. Thus rhamnose gives a green color with reagent r, but other methylpentoses have not been tried. Mannose gives a violet to purple color with reagent v and also with w. Fructose, heated with a dilute solution of cobaltous chloride, cooled, and then treated with ammonia, produces a violet to purple color. Maltose gives a blue color with reagent k, and red to brown with reagent x. Starch is colored blue by iodine, dextrin reddish violet to reddish brown, and glycogen wine-red. Pectin colors a cold 5 per cent solution of potassium hydroxide bright yellow, and produces the color of bichromate with a solution of sodium chromate. In most cases it is necessary to confirm the identification with one reagent by the application of others.

[164] *Ind. Eng. Chem., Anal. Ed.*, **4, 413 (1932).**

TABLE XCVI. REAGENTS

	Reagent	Solvent	Amt. of Reagent	Time Heated at 100° C.	Elapsed Time Reading	Remarks
			per cent	*Min.*	*Min.*	
a	Water	[a]				Cellulose insoluble
b	I + KI	H_2O	3	..	..	Heat to expel free iodine; cool
c	Nylander's[b]	8% NaOH	6	1	1–5	Reagent catalyzed with $AuCl_3$; (+) bk. ppt's.; (wh) white ppt's.
d	Disch's	[c]		30	30+	Pentoses brown; hexoses, etc., blue
e	Orcinol	[d]		1	1–5	See Table XCVII
f	Phloroglucinol	[e]		1	1–5	See Table XCVII; boiling gives ChR with pectin, gum arabic, and gum tragacanth
g	Na_2CrO_4	H_2O	10	1	1–60	Simple sugars green; pectin, red solution
h	Sodium nitroprusside	H_2O	1	1+	?	Add NH_4OH and heat; (+) green while hot; arabinose green without NH_4OH
i	$KMnO_4$	H_2O	0.1	..	1–60	(+) immediate decolorization; (*) decolor. in 1 hr.
j	Fehling's[f]	H_2O		..	60	
k	$K_3Fe(CN)_6$	H_2O	0.5	1	1–5	$+FeCl_3$; brown, negative; greens, positive; maltose, blue
l	Picric acid	H_2O	Sat.	1	1–5	$+NH_3$; nos. indicate depth of colors (red)
m	H_3PO_4	H_2O	85	1	1–60	Yellow to amber to brown to black; nos. = depth of colors
n	$HClO_4$	H_2O	60	1	1–60	Yellow to amber to brown to black; nos. = depth of colors
o	H_2SO_4	H_2O	50	1	1–60	Yellow to amber to brown to black; nos. = depth of colors
p	HCl	H_2O	20	1	1–60	Yellow to amber to brown to black; nos. = depth of colors
q	KOH[g]	H_2O	5	1	1–60	Nos. = depth of color; pectin, yellow
r	$(NH_4)_2MoO_4$	H_2O	5	..	60	Add carbohydrate, then 1/2 the vol. conc. H_2SO_4. Nos. = depth of color; rhamnose, green
s	HNO_3	H_2O	35	..	..	Evap. 2/3 for mucic acid
t	H_2SeO_3	50% H_2SO_4	10	1	1–60	Nos. = depth of red
u	Ni_2O_3	[h]		..	..	Nos. = relative speeds of decolorization of Ni_2O_3
v	Heroine	H_2SO_4	1	..	15	Mostly ambers to brown-blacks[i]
w	Codeine	H_2SO_4	1	..	15	Mostly rose to brown-blacks[i]
x	$AgNO_3$	Dil. NH_4OH	1	1	1–60	(+) black ppt. or silver mirror; some give brown solution
y	*p*-Tolylhydrazine	Dil. HCl	1	..	1–60	Boiling gives for (+) dichromate color; levulose and sucrose give color and ppts.

[a] Mono-, di-, and trisaccharides are sweet and soluble in cold water. Polysaccharides, except cellulose, dissolve in hot water. Starch, pectin, glycogen, and gum tragacanth dissolve in hot water to give opalescence and foam. Gum arabic gives clear solution and foam. Dextrin and inulin give clear solutions but no foam.

[b] Nylander's reagent: 2 g. of bismuth subnitrate and 4 g. of Rochelle salt dissolved in 100 g. of 8% NaOH.

[c] Prepare 10% solution of diphenylamine in alcohol and add 1 volume to 4 volumes of acetic acid and 5 volumes of HCl.

[d] To 5 ml. of saturated aqueous solution of orcinol, add 50 ml. of HCl and dilute to 90 ml.

[e] To 0.2 g. of phloroglucinol in 10 ml. of alcohol, add 50 ml. of HCl and dilute to 90 ml. Solution of orcinol or of phloroglucinol in dilute HCl will indicate carbohydrates containing levulose.

[f] Fehling's reagent: (1) solution of 36.44 g. of $CuSO_4 \cdot 5H_2O$ in water diluted to 500 ml.; (2) solution of 125 g. of KOH and 173 g. of sodium potassium tartrate in water diluted to 500 ml.

[g] Usually called Moore's test.

[h] Prepare solutions (*a*) 10 g. of $NiSO_4 \cdot 7H_2O$ in 1000 ml. of H_2O, and (*b*) 4.8 g. of $K_2S_2O_8$ and 6 g. of KOH in 1000 ml. of H_2O. Use equal parts and let stand — black Ni_2O_3 precipitates. Carbohydrates decolorize this.

[i] With varied times and temperatures carbohydrates give changing colors. A check with a known carbohydrate is revealing and desirable. Perhaps in these reactions, formaldehyde is split off, because colors closely follow Kobert's test for codeine, etc.

TABLE XCVII

Effects of Various Reagents on Carbohydrates

Carbohydrate	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y
1 Cellulose	−	−	−	Bl	−	−	−	−	−	−	Br	−	−	−	−	−	−	3	−	−	−	R	r	−	−
2 Starch	+	+	Wh	Bl	−	−	−	−	*	−	Br	−	−	−	1	−	−	−	−	−	−	−	r	−	−
3 Soluble starch	+	+	Wh	Bl	−	−	−	−	−	−	Br	−	rose	−	4	−	−	−	−	5	−	−	−	−	−
4 Glycogen	+	+	Wh	Bl	−	−	−	−	+	−	G	−	1	1	1	3	−	3	−	−	1	−	rV	Br	−
5 Dextrin	+	+	Wh	Bl	−	Am	−	−	+	+	G	−	−	−	2	−	5	5	−	1	3	−	−	Br	+
6 Pectin	+	−	−	Br	−	−	R	+	*	+	G	−	−	1	2	−	Y	−	+	1	1	−	−	Br	+
7 Levulose	+	−	+	Bl	Br	Br	+	+	*	+	G	9	9	9	9	9	9	9	−	9	4	Br	Br	+	+
8 Mannose	+	−	+	Bl	−	Bk −	+	+	*	+	G	2	1	−	3	1	7	−	−	5	7	Bk V	Bk VP	+	−
9 Maltose	+	−	+	Bl	−	Y	+	+	+	+	Bl	2	3	1	5	−	8	6	−	3	9	Am	r	r − Br	+
10 Rhamnose	+	−	+	Br	−	Or	+	+	−	+	G	2	2	3	6	−	4	G	−	8	4	OrR	R	+	−
11 Arabinose	+	−	+	Br	Bl	ChR	+	+	*	+	G	9	1	2	2	−	7	7	−	3	8	r	R	+	−
12 Xylose	+	−	+	Br	Bl	ChR	+	+	*	+	G	9	1	−	7	−	7	5	−	7	8	R	R	+	−
13 Galactose	+	−	+	Bl	−	Am	+	+	+	+	G	2	1	−	3	−	7	−	+	6	5	−	−	+	−
14 Lactose	+	−	+	Bl	−	Am	+	+	−	+	G	2	−	−	3	−	7	6	+	6	2	−	−	+	−
15 Raffinose	+	−	−	Bl	Br	Br Bk	−	−	−	−	Br	−	3	2	6	5	−	7	+	8	2	Am Bk	Am Bk	−	+
16 Sucrose	+	−	−	Bl	Br	Br Bk	−	−	−	−	Br	−	8	8	8	7	−	8	−	8	2	Br Bk	Am Bk	−	+
17 Inulin	+	−	−	Bl	Br	Br	−	−	*	+	G	−	8	7	9	6	−	8	−	5	2	Am	r	−	+
18 Glucose	+	−	+	Bl	−	Y	+	+	*	+	G	2	−	−	3	−	8	−	−	2	6	−	−	+	+
19 Gum arabic	+	−	Am	Bl Bk	−	−	−	−	+	+	G	−	3	2	6	−	3	2	+	5	1	r	−	−	−
20 Gum tragacanth	+	−	Wh	Br Bk	−	−	−	−	+	−	G	−	rose	1	4	−	−	4	+	6	1	R	R	−	−
21 Agar agar	+	−	Am	Bl	Br	Br Bk	−	−	−	−	G	−	−	4	4	3	5	8	+	−	−	Br R	VR	−	+

Abbreviations: Am = amber; Bk = black; Bl = blue; Br = brown; Ch =cherry; G = green; Or = orange; P = purple; R = red; r = rose; V = violet; Wh = white; Y = yellow.

Identification of Sugars by Color Reactions, according to Dische.[165] In this scheme α-naphthol in sulfuric acid, diphenylamine in hydrochloric acid, indole in sulfuric acid, and phloroglucinol in sulfuric acid are used in various combinations for the qualitative detection of various sugars and related substances, and also for approximate quantitative work on simple mixtures. Dische presents his method in tabular form as follows:

TABLE XCVIII

Reagent	Color Reaction
Naphthol Reagent I: 1 ml. sugar solution, 9 ml. sulfuric acid, made of 8 ml. concentrated acid and 1 ml. water. Place mixture for 3 minutes in boiling-water bath. Cool, add 0.2 ml. of 5 per cent α-naphthol solution. Wait 10 minutes.	Pentoses, hexoses, glucuronic acid red-violet. Glycolic aldehyde, trioses, lactic acid brown.
Naphthol Reagent II: 1 ml. sugar solution, 0.1 ml. of 2 per cent α-naphthol solution, plus 9 ml. of 75 per cent sulfuric acid. Wait 15 minutes.	Aldopentoses and aldohexoses pink; fructose blue. Trioses violet, solution turning turbid on standing. Aldehydes brown. Glucuronic acid no color.
Naphthol Reagent III: 1 ml. sugar solution, 0.1 ml. of 10 per cent α-naphthol solution, plus 8 ml. concentrated sulfuric acid, without cooling.	All carbohydrates red, glucuronic acid brown.
Diphenylamine Reagent I: 2 ml. sugar solution, plus 4 ml. of a solution made of 100 ml. concentrated hydrochloric acid, 80 ml. glacial acetic acid, and 20 ml. of 10 per cent solution of diphenylamine in alcohol. Heat for 30 minutes in boiling-water bath.	Hexoses blue. Pentoses brown. Glucuronic acid, aldehydes, and thymonucleic acid violet-red.
Diphenylamine Reagent II: Same as diphenylamine reagent I, but heat only 1.5 minutes.	Glucose and fructose blue. Galactose violet. Mannose brown.
Diphenylamine Reagent III: Same as diphenylamine reagent I, but heat for 3 minutes.	Fructose brown. Glucose and galactose in 1 per cent solution no color.
Indole Reagent: 1 ml. sugar solution plus 9 ml. 75 per cent (by volume) sulfuric acid, plus 0.3 ml. of 1 per cent solution of indole in alcohol. Heat for 10 minutes in boiling-water bath.	Pentoses, hexoses, glucuronic acid brown.
Phloroglucinol Reagent: 1 ml. sugar solution, plus 5 ml. of 60 per cent (by volume) sulfuric acid, plus 0.1 ml. of 10 per cent alcoholic phloroglucinol solution. Heat 2 to 3 minutes in boiling-water bath.	Pentoses during first minute red, then turning brown. Hexoses brown from beginning. Aldehydes brown without heating.

TABLE XCIX

NAPHTHOL REAGENT III

Red: Glycolaldehyde, trioses, pentoses, hexoses, thymonucleic acid.	*Brown:* Glucuronic acid (gives red color with naphthol reagent I, brown with indole reagent).

[165] *Mikrochemie*, 7, 33 (1929).

NAPHTHOL REAGENT I

Brown		*Red*
Glycolaldehyde	Trioses	
1 ml. sugar solution plus 0.1 ml. of 10 per cent α-naphthol solution, plus 4 ml. concentrated sulfuric acid, without cooling: Greenish blue, other carbohydrates red.	1 ml. sugar solution, plus 4 ml. concentrated sulfuric acid. Cool, add 0.1 ml. of 10 per cent α-naphthol solution: Green, other carbohydrates red or brown.	Pentoses, hexoses, thymonucleic acid.

DIPHENYLAMINE REAGENT I

Brown: Pentoses	*Blue:* Hexoses	*Violet-red:* Thymonucleic acid

NAPHTHOL REAGENT II

Pink: Aldoses; coloring matter precipitated upon dilution with water.	*Blue:* Fructose, hexosediphosphoric acid; coloring matter not precipitated upon dilution with water.

DIPHENYLAMINE REAGENT II

Violet: Galactose	*Brown:* Mannose	*Blue:* Glucose

DIPHENYLAMINE REAGENT III

Brown: Fructose	*Violet:* Hexosediphosphoric acid

By the use of the reagents given in Table XCIX, in regular order, the various carbohydrates listed may be distinguished from one another.

TABLE C

RELATIVE DEPTH OF COLOR PRODUCED, ON THE BASIS OF 1 FOR GLUCOSE

	Mannose	Galactose	Fructose
Naphthol I	0.4	0.5	1.30
Naphthol II	10	5	Blue
Diphenylamine I	1.1	0.6	5 (about)
Diphenylamine II	1	less than 0.6	50
Indole	0.7	0.75	1.25
Phloroglucinol,			
2 minutes' heating	3.0	3.30	12.00
3 minutes' heating	2.60	....	.....

In the case of mixtures of two or three of the hexoses listed in Table C, the relative quantities of each may be estimated approximately by using two or three of the reagents, measuring the color in a colorimeter, and solving the resulting equations for each of the constituents present in the same manner as is customary in indirect analysis.

Identification by Fermentation with Yeasts. The action of different yeasts on various sugars, as given by Fischer and Thierfelder,[166] is shown in Table CI.

TABLE CI

ACTION OF YEASTS UPON DIFFERENT SUGARS

	d-Mannose	*d*-Fructose	*d*-Galactose	Sorbose	*l*-Arabinose	Rhamnose	Sucrose	Maltose	Lactose
S. Pastorianus I	+++	+++	+++	0	0	0	+++	+++	0
S. Pastorianus II	+++	+++	++	0	0	0	+++	+++	0
S. Pastorianus III	+++	+++	+++	0	0	0	+++	+++	0
S. cerevisiae I	+++	+++	+++	0	0	0	+++	+++	0
S. ellipsoideus I	+++	+++	++	0	0	0	+++	+++	0
S. ellipsoideus II	+++	+++	+	0	0	0	+++	+++	0
S. Marxianus	+++	+++	+++	0	0	0	+++	0	0
S. membranaefaciens	0	0	0	0	0	0	0	0	0
Brewery yeast	+++	+++	+++	0	0	0	+++	+++	0
Distillery yeast	+++	+++	+	0	0	0	+++	+++	0
S. productivus	+++	+++	0	0	0	0	+	+++	0
Milk-sugar yeast	++	+++	+	0	0	0	+++	0	+++

+++ = complete fermentation after 8 days.
++ = almost complete fermentation after 8 days.
+ = only partial fermentation after 8 days.
0 = no fermentation after 8 days.

Kluyver[167] found that all yeasts which ferment glucose also ferment fructose and mannose, and all those that ferment sucrose also ferment raffinose. All yeasts that ferment lactose have no effect on maltose. Galactose and sucrose are fermented by *Saccharomyces cerevisiæ* and by lactose yeast, but not by *Saccharomyces apiculatus, Torula monosa,* or *Torula fluorescens. Torula dattila, Schizosaccharomyces pombe,* and *Saccharomyces variabilis* ferment sucrose, but not galactose. While some species of yeasts are thus valuable for differentiating between certain sugars, their use for identifying sugars in mixtures is rather limited.

Identification of Carbohydrates by Means of Bacteria. Kendall and coworkers[168] found that carbohydrates can be identified more quickly and reliably by means of bacteria than of yeasts, and that even the quantity of carbohydrates present can be determined with fair accuracy. The nutrient medium consists of an aqueous meat extract prepared in the usual manner, freed from muscle sugar, and reinforced

[166] *Ber.*, 27, 2031 (1894).
[167] Dissertation, Delft, 1914.
[168] *J. Infectious Diseases*, 32, 355, 362, 369, 377 (1923).

with 0.5 per cent peptone. The reaction is adjusted to *p*H 6.8 with phosphate buffer. Sterility of the medium is tested by incubation. Four milliliters of the medium is placed in a test tube, and 1 ml. of the sugar solution which has been sterilized by passing through a stone filter is added. Sterilization by heat must be avoided because it may

TABLE CII

Identification of Carbohydrate with Bacterial Reagents

Microbic Reagent	Sugar					
	Glucose	Fructose	Mannose	Galactose	Lactose	Saccharose
B. proteus	+	−	−	+	−	+
Mic. tetragenus	+	+	−	+	−	−
B. mesentericus	+	+	−	−	−	−
Vibrio cholerae	+	+	−	+	+	+
Vibrio of Finkler and Prior	+	+	+	−	−	+
B. typhosus	+	+	+	+	−	−
B. coli I	+	+	+	+	+	−
B. coli II	+	+	+	+	+	+

The sign + indicates an increase in the hydrogen-ion concentration of the medium, and therefore fermentation (utilization for energy) of the sugar. The sign − indicates an increase in the hydroxyl-ion concentration, due to action on the protein constituents of the medium for energy, and therefore no fermentation.

TABLE CIII

Reactions of Organisms Studied in Various Mediums

	d-Glucose	Gluconic Acid	Saccharic Acid	Sorbitol	Fructose	Mannose	Mannonic Acid	Mannosaccharic Acid	Mannitol	Galactose	Galactonic Acid	Mucic Acid	Dulcitol	
Staphyl. aureus	+	+	−	∓	+	+	−	−	∓	∓	−	−	−	6 strains
Mic. tetragenus	+	+	−	−	+	+	−	−	+	∓	−	−	−	2 strains
Mic. zymogenes	+	+	+	+	+	+	+	−	+	+	+	−	−	
Mic. ovalis	+	+	+	+	+	+	+	−	+	+	+	−	−	3 strains
Str. pyogenes	+	+	+	+	+	+	+	−	+	+	+	−	−	4 strains. See, Kendall, Day, Walker and Ryan
Pneumococcus	+	+	+	+	+	+	+	−	+	+	+	−	−	Types I–IV, inclusive
B. dysenteriae, Shiga	+	+	+	+	+	+	∓	−	+	+	+	−	−	8 strains
B. dysenteriae, Flexner	+	+	+	+	+	+	∓	−	∓	+	+	−	−	
B. dysenteriae, Somme	+	+	+	+	+	+	+	−	+	+	+	−	−	3 strains
B. typhosus	+	+	+	+	+	+	+	−	+	+	+	−	−	2 strains
B. paratyphosus, alpha	g	g	g	g	g	g	+	−	g	g	g	−	−	
B. paratyphosus, beta	g	g	g	g	g	g	−	−	g	g	g	−	−	3 strains
B. coli	g	g	g	g	g	g	+	−	g	g	g	−	−	
B. proteus	g	g	g	−	−	−	−	−	−	g	g	−	−	5 strains
B. mucosus capsulatus	g	g	g	g	g	g	g	−	g	g	g	..	g	6 strains
Vibrio cholerae	+	+	+	−	+	∓	∓	−	+	+	+	−	−	4 strains

\+ = acid reaction; g = gas and acid; − = no fermentation, reaction becomes gradually alkaline; ± = majority but not all strains give an acid reaction; ∓ = majority but not all strains fail to ferment.

bring about chemical changes in the sugar. A control tube is prepared, containing 1 ml. of sterile water. The tubes are inoculated with a culture which has been transferred on three successive days. After 4 hours' incubation at 37° C. the reaction is determined colorimetrically

with bromthymol blue and bromcresol purple. The effect of several bacteria on various sugars is shown in Table CII.

The list of carbohydrates and of bacteria is extended in Table CIII, where gas formation is also used as a criterion.

If the *p*H of the solution is compared with that obtained under the same conditions with various dilutions of the sugar present, the approximate concentration in the unknown can be determined.

TABLE CIV

	Glucose	Fructose	Maltose	Galactose	Sucrose	Lactose	Mannitol	Dulcitol	Dextrin	Raffinose	Arabinose	Adonitol	Inulin	Sorbitol	Starch	Glycerol	Inositol	Salicin	Amygdalin	Isodulcitol	Erythritol
Monilia baleanica	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M. krusei	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M. pinoyi	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M. metalondinensis	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M. tropicalis	G	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M. rhoi	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M. macedoniensis	G	G	G	G	G	G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacillus coli Esch.	G	G	G	G	G	G	G	G	G	G	G	0	0	G	0	G	0	G	0	G	0
B. pseudocoli	G	G	G	G	0	0	G	G	G	0	G	0	0	G	0	0	0	0	0	0	0
B. paratyphosus B, var. M	G	G	G	G	0	0	G	G	G	0	G	0	0	G	0	G	0	0	0	G	0
B. paratyphosus A, Schottm.	G	G	G	G	0	0	G	G	G	0	G	0	0	G	0	G	0	0	0	G	0
B. asiaticus	G	G	G	G	G	0	G	G	G	G	G	0	0	G	0	G	0	0	0	G	0
B. pseudoasiaticus	G	G	G	G	G	0	G	G	G	G	G	0	0	G	0	G	0	0	0	G	0

G = gas formation; 0 = no gas formation. Acid formation is not taken into account.

Identification by Fermentation with Fungi and Bacteria. This method is due to Castellani and Taylor.[169] The technique is as follows. A sterile, 1 per cent solution of the sugar in peptone water, which must be free from sugar, is prepared, and the solution distributed in two or more fermentation tubes of the Durham or similar type. Each tube is inoculated with a pure culture of one of the fungi and bacilli listed in Table CIV above. The tubes are incubated at 37° C. for 3 to 4 days. The development of gas, or its absence, is used as the criterion for identifying the sugar. Galactose, for instance, forms gas with *Monilia tropicalis*, but not with *Monilia baleanica*; sucrose gives a positive result with *Bacillus asiaticus*, but not with *Bacillus coli*.

The fermentation test may also be combined with copper-reduction and other tests.

Method of Harding and Nicholson. The system just described has been modified and further developed by Harding and Nicholson.[170]

[169] *Biochem. J.*, 16, 657 (1922).

[170] *Biochem. J.*, 27, 1082 (1933); 30, 1804 (1936).

Only four organisms are used, and with these it is possible to detect, and to determine the approximate quantity of, sucrose, maltose, lactose, glucose, galactose, fructose, and mannose, in mixtures of these sugars. Not more than 10 mg. of each sugar should be present in 100 ml. solution. Clarification of impure solutions may be effected with lead acetate followed by primary potassium phosphate, or primary potassium phosphate followed by magnesium oxide, or mercuric sulfate and barium carbonate followed by hydrogen sulfide. The *p*H is always adjusted to 6.5 to 7, the culture added, and the mixture incubated. The copper-reducing power of the solution is determined before and after each treatment. Each culture must first be tested with the individual sugar to be detected, in order to determine the amount of culture and the incubation period to be employed. The systematic procedure is as follows:

A. A portion of the original solution is treated with *Monilia krusei.* This ferments glucose, fructose, and mannose.

B. In the residue from A galactose is detected by means of *Saccharomyces marxianus.*

C. In another portion of the original solution the glucose and fructose are fermented by *Gaffkya tetragena.* A–C gives the mannose.

D. If galactose is found to be absent by test B, another portion of the original solution is treated with *Proteus vulgaris,* which is specific for glucose. C–D gives the fructose.

E. In the presence of galactose, as disclosed by test B, the residual solution from C is treated with *Monilia krusei,* and the sum of fructose and mannose is obtained. The fructose is obtained by the difference between C and the glucose, or E and the mannose.

F. The residual solution from either A or E is hydrolyzed as in the Clerget procedure with hydrochloric acid, and the resulting glucose and fructose found by the use of *Proteus vulgaris* and *Monilia krusei.* This gives a measure of the sucrose.

G. The original solution is treated with *Saccharomyces marxianus* followed by *Monilia tropicalis.* This gives the maltose.

H. The residual solution from G is hydrolyzed with hydrochloric acid, and then tested for galactose as under B. Lactose is thus detected.

Bourquelot's Biochemical Method for Detecting Compound Sugars and Glucosides.[171] This method is based on the observation of Bourquelot that, when an enzyme acts upon a glucoside or higher (di-, tri-, or tetra-) saccharide, there exists a definite relationship between the change in reducing power and the change in rotation. This ratio

[171] See Béguin's review in *Pharm. Acta Helv.*, 1, 65, 90 (1926).

is characteristic for each compound sugar or glucoside, and is termed the "enzymolytic index of reduction." The principal enzymes used by Bourquelot and his coworkers are invertase, emulsin, and rhamnodiastase. The method has been used particularly for the identification of higher saccharides and glucosides in plant materials.

The plant material to be tested is first boiled under reflux with 95 per cent alcohol for 30 minutes, to destroy any enzymes present and to extract the glucoside. The treatment is repeated to complete the extraction. The alcoholic extract and washings are evaporated under vacuum at a temperature below 50° C., and the dry residue is dissolved in water. The solution is clarified with lead subacetate, the excess lead removed with hydrogen sulfide, and the filtrate made up to a definite volume. In a part of the solution the reducing power is determined by Bertrand's method, and also the optical rotation. Another part of the solution is treated with invertase at 30 to 35° C., a few drops of toluene being added. The reducing power and rotation are redetermined from time to time. If a fructoside, such as sucrose, raffinose, or stachyose, is present, the reducing power increases, and the dextrorotation decreases. When both have become constant, the enzymolytic index of reduction is calculated, dividing the milligrams reducing sugar formed under the influence of invertase, in terms of glucose, by the change in rotation. The same ratio is determined under the same conditions for various sugars which may be expected to be present, and the results are compared. If the enzymolytic index of the unknown material coincides with that of a known sugar, further confirmatory tests may be applied; if not, the material may contain a mixture of sugars or a new sugar.

The invertase in the solution is now destroyed by boiling the neutralized solution for 10 minutes, and the solution is treated with emulsin. If any compounds hydrolyzed by this agent are present, the reducing power will increase further, and the rotation will change toward the right. The enzymolytic index is calculated and compared with that of known compound sugars or glucosides. After destroying the emulsin by boiling, still other enzymes may be applied.

In this manner Bourquelot and his pupils have established the presence of sucrose, raffinose, and stachyose in a number of plants, where acid hydrolysis would not have given the desired result. The tetrasaccharide verbascose was discovered by this method, and a series of new glucosides were found by the use of emulsin or of rhamnodiastase.

CHAPTER XIV

REDUCTION METHODS FOR DETERMINING SUGARS

The principal chemical methods for determining sugars are based upon the property which all aldehydes and ketones have of reducing alkaline solutions of certain metallic salts. The reducing action of glucose, lactose, and other sugars upon alkaline solutions of copper, silver, mercury, bismuth, and other metals has already been mentioned. In the case of silver and glucose, for example, the reaction when carefully controlled proceeds as follows:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 9\ \underset{\text{Silver oxide}}{Ag_2O} \longrightarrow 18\ \underset{\text{Silver}}{Ag} + 3\ \underset{\text{Oxalic acid}}{(COOH)_2} + 3\ \underset{\text{Water}}{H_2O}$$

If the weight of reduced silver be determined for this reaction, the amount of glucose can easily be estimated. But unfortunately the reducing action of sugars upon metallic salts does not proceed with the quantitative precision of the above equation; the reduction is rarely complete, and the amount of reduced metal varies with the conditions of the experiment. The latter difficulty is obviated, however, in practice by controlling the process so that the same weight of reduced metal is always obtained for the same weight of sugar.

Of the various alkaline solutions of metals those of copper are employed most generally in sugar analysis.

COPPER–REDUCTION METHODS

Early Methods.[1] The reducing action of sugars upon different salts of copper has been known since the beginning of chemistry. Trommer,[2] in 1841, first noted the value of alkaline copper sulfate solution as a means of distinguishing grape from cane sugar. Trommer's method was improved in 1844 by Barreswil,[3] who made the important discovery that addition of potassium tartrate to the alkaline copper sulfate solution greatly increased its stability. Barreswil's method was vol-

[1] For the history of copper-reduction methods see Herstein, *J. Am. Chem. Soc.*, 32, 779 (1910); Cattelain, *J. pharm. chim.*, [8], 10, 405, 449 (1929).

[2] *Ann.*, 39, 360 (1841).

[3] *J. pharm. chim.*, [3], 6, 301 (1844).

umetric; the sugar solution was slowly added to the boiling copper reagent, which had previously been standardized against pure glucose, until the blue color was just discharged.

Fehling's Method. Fehling,[4] in 1848, first worked out the details of the alkaline copper method, as they now stand, and the copper sulfate and alkaline tartrate reagent has since been called by his name.

The copper solution employed by Fehling consisted of 40.00 g. copper sulfate, $CuSO_4 \cdot 5H_2O$, 160 g. neutral potassium tartrate, and 600–700 g. sodium hydroxide solution of 1.12 sp. gr. dissolved in water to 1154.4 ml. This is equivalent to 34.65 g. $CuSO_4 \cdot 5H_2O$ dissolved to 1000 ml., the proportion used by nearly all subsequent workers down to the present time.

Fehling's solution contains 8.822 g. copper to 1000 ml. or 0.008822 g. to 1 ml. According to Fehling's experiments 1 ml. of his solution was exactly reduced by 0.005 g. of anhydrous glucose, or 1 part glucose reduced 1.765 parts copper. In terms of the molecular weight of glucose the ratio would be $180 \times 1.765 = 317.6$. Dividing this value by 63.6, the atomic weight of copper, the atoms of copper reduced by one molecule of glucose is found to be five. The reduction ratio 1 : 5 was regarded as constant by Fehling and was so employed by all chemists until Soxhlet[5] showed in 1878 that the ratio between sugar and amount of copper reduced was not a constant but varied according to the excess of copper which is present during the reaction.

Nature of Fehling's Solution. Many chemists consider Fehling's and other alkaline copper solutions to contain complex copper salts of definite composition, some of which have been described in the literature. But the behavior of these reagents suggests, as has been pointed out by Dumanskii[6] and by others, that they are sols of $Cu(OH)_2$ in a high degree of dispersion. This would explain the ageing of Fehling's solution, the formation of Cu_2O sols under certain conditions, and similar colloid-chemical phenomena observed in the use of these solutions.

The more modern methods of sugar determination which employ Fehling's solution may be divided into two general classes. I. Volumetric methods based upon the complete reduction of a measured volume of standard solution. II. Methods in which an excess of copper reagent is employed, and either the reduced or the unreduced copper determined, with or without previous filtration. In some methods the reduced copper is estimated gravimetrically, in others the reduced or unreduced copper is determined by titration or colorimetrically.

[4] *Ann.*, **72**, 106 (1849); **106**, 75 (1858).
[5] *J. prakt. Chem.*, [2], **21**, 227 (1880).
[6] *Kolloid Z.*, **47**, 121 (1929).

Volumetric Methods Based upon the Complete Reduction of a Measured Volume of Fehling's Solution

Soxhlet's Method. Owing to the decomposition which takes place in the mixed copper sulfate and alkaline tartrate solution upon standing, the two solutions employed in the Soxhlet and other modern methods are mixed only just before using. The solutions consist of the following: *Solution A*, 34.639 g. of pure crystallized $CuSO_4 \cdot 5H_2O$ is dissolved in water and made up to 500 ml. *Solution B*, 173 g. of Rochelle salts and 50 g.[7] NaOH are dissolved in water, the volume is completed to 500 ml., and the solution allowed to stand for 2 days. Both solutions A and B are filtered separately through purified asbestos. Previous to analysis equal volumes of solutions A and B are mixed.

Before using the mixed copper reagent, it should be standardized against glucose, invert sugar, lactose, etc., according to the needs of analysis. Since reducing sugar in sugar cane, sugar beet, and most other food products is most usually expressed as invert sugar, the latter is most commonly used for standardization. A standard solution of invert sugar has also an advantage in being easily prepared.

Standard Invert Sugar Solution.[8] Dissolve 4.75 g. of pure sucrose in 75 ml. of water, add 10 ml. of hydrochloric acid (*d* 1.1029 at 20°/4°), and set aside during a period of 24 hours at a temperature not below 20° C. Neutralize the acid exactly with dilute sodium hydroxide and make up to 1000 ml.; 100 ml. of this solution contains 0.500 g. of invert sugar.

According to Lane and Eynon[9] it is better not to neutralize the acid before making up to volume, because the acid solution will keep for long periods of time without change in titer, whereas the neutralized solution will quickly deteriorate. The aliquots used for standardization are neutralized immediately before use.

The amount of standard invert sugar solution necessary to reduce 100 ml. of the mixed copper reagent is determined according to the details described in the next paragraph.

Application to Analysis of Sugar Products.[10] Make a preliminary titration to determine the approximate percentage of reducing sugar in the material under examination. Prepare a solution which contains approximately 1 per cent of reducing sugar. Place in a beaker 100 ml. of the mixed copper reagent and approximately the amount of the sugar

[7] Soxhlet specified 51.6 g., but this has been rounded off to 50 g. in practically all modern books of methods.

[8] "Methods of Analysis, A. O. A. C.," 3d ed., p. 377, 1930.

[9] *J. Soc. Chem. Ind.*, **42**, 32T (1923).

[10] "Methods of Analysis, A. O. A. C.," 2nd ed., p. 190, 1925.

solution for its complete reduction. Boil for 2 minutes. Filter through a folded filter and test a portion of the filtrate for copper by use of acetic acid and potassium ferrocyanide. Repeat the test, varying the volume of sugar solution, until two successive amounts are found which differ by 0.1 ml., one giving complete reduction and the other leaving a small amount of copper in solution. The mean of these two readings is taken as the volume of the solution required for the complete precipitation of 100 ml. of the copper reagent.

Under these conditions 100 ml. of standard copper reagent requires 0.475 g. of anhydrous glucose or 0.494 g. of invert sugar for complete reduction. Calculate the glucose by the following formula:

V = the volume of the sugar solution required for the complete reduction of 100 ml. of standard copper reagent.
W = the weight of the sample in 1 ml. of the sugar solution.

Then $$\frac{100 \times 0.475}{V \times W} = \text{per cent of glucose}$$

or $$\frac{100 \times 0.494}{V \times W} = \text{per cent of invert sugar}$$

In making the test for unreduced copper a few drops of the filtered solution are placed upon a white test plate, acidified with a few drops of 10 per cent acetic acid, and a drop of 2 per cent potassium ferrocyanide solution is added. A brown coloration indicates the presence of unreduced copper.

Volume of Fehling's Solution Reduced by Different Sugars. The ratio between volume of standard Fehling's solution and the amount of different sugars just sufficient to cause complete reduction was determined by Soxhlet[11] to be as follows:

TABLE CV

Volume of Fehling's Solution Reduced by Different Sugars	Reducing Power in Terms of Glucose
0.5000 g. glucose reduces 105.2 ml. Fehling's solution	1.000
0.5000 g. invert sugar reduces 101.2 ml. Fehling's solution	0.962
0.5000 g. fructose reduces 97.2 ml. Fehling's solution	0.924
0.5000 g. lactose reduces 74.0 ml. Fehling's solution	0.703
0.5000 g. maltose reduces 64.2 ml. Fehling's solution	0.610

The above results calculated to equal volumes of copper reagent show that 100 ml. of mixed standard Fehling's solution is reduced by

[11] *J. prakt. Chem.*, [2] 21, 227 (1880).

0.4753 g. of glucose, 0.4941 g. of invert sugar, 0.5144 g. of fructose, 0.6757 g. of lactose, and 0.7788 g. of maltose.

Modifications of Soxhlet's Method. Instead of employing 100 ml. of Fehling's solution for the Soxhlet determination, it is more customary to use 10 ml., 20 ml., or 50 ml., the quantity thus taken being measured into a casserole, beaker, or flask, and diluted, according to requirements, with a measured volume of water. For very dilute sugar solutions, as small a quantity as 5 ml. of Fehling's solution may be used to advantage.

In any of the numerous modifications of Soxhlet's method, it is important that the Fehling solution be standardized under exactly the same conditions as in analysis. The same degree of dilution should be followed for the mixed copper reagent in all experiments. Soxhlet found that 0.5 g. of glucose reduced 105.2 ml. of Fehling's solution when undiluted and only 101.1 ml. when diluted with 4 parts of water; similar results were also obtained with other sugars. Such differences as these might produce a variation of several per cent in the estimation of reducing sugars.

It is also evident that to obtain the most concordant results the sugar solutions should always contain about the same percentage of reducing sugar. This is accomplished in practice by making a rough preliminary determination and then making up a fresh sugar solution so that the percentage of reducing sugar shall be 0.1 per cent, 0.5 per cent, or 1.0 per cent, etc., according to the volume of Fehling's solution taken and the individual preference of the chemist. In this manner approximately the same volume of sugar solution is always used for reducing the same volume of copper reagent, and under such conditions, with a uniform method of boiling, the most accurate results are obtained.

A difference in reducing power is also obtained whether the sugar solution is added to the copper reagent in small portions, with successive periods of boiling, or only in one portion with one period of boiling. The most accurate results are secured where the test is made with the entire volume of sugar solution necessary for complete reduction, with only one period of boiling.

The following example will give an illustration of the application of the method:

Example. Twenty milliliters of Fehling's solution diluted with 80 ml. of water was found to require for reduction exactly 20.2 ml. of standard invert sugar solution or 0.101 g.

Fifty grams of sugar-cane molasses was diluted to 1000 ml. Of this solution about 8 ml. was required to discharge the blue color of 20 ml. Fehling's solution.

Eighty ml. of the sugar solution (4 g. molasses) was then made up to 200 ml. (1 ml. = 0.02 g. molasses). Of this solution 19.6 ml. when boiled with 20 ml. Fehling's solution and 80 ml. of water for 2 minutes showed incomplete reduction by the ferrocyanide test and 19.8 ml. complete reduction.

Calling 19.7 ml. the volume of sugar solution necessary to reduce the 20 ml. of Fehling's solution, then $\frac{0.101 \times 100}{0.02 \times 19.7} = 25.64$ per cent invert sugar in the molasses.

The Ferrocyanide Test for Copper. Several methods are followed for making the ferrocyanide test for unreduced copper. It sometimes happens that the cuprous oxide is precipitated in a very finely divided form and gives annoyance by running through the filter.

One method of making the test is to superimpose several small strips of filter paper and allow a few drops of the solution to fall upon the upper paper. The moistened area upon the second or third underlying strip is then treated with a drop of ferrocyanide solution acidified with acetic acid. The appearance of a brown spot indicates the presence of unreduced copper.

Method of Ross. A method due to Ross[12] is to dip the point of a small folded filter, held by means of forceps, below the surface of the hot solution in the casserole and withdraw a few drops of the clear liquid from the interior of the filter by means of a medicine dropper (Fig. 273). The method is simple and particularly useful where there is a large amount of routine.

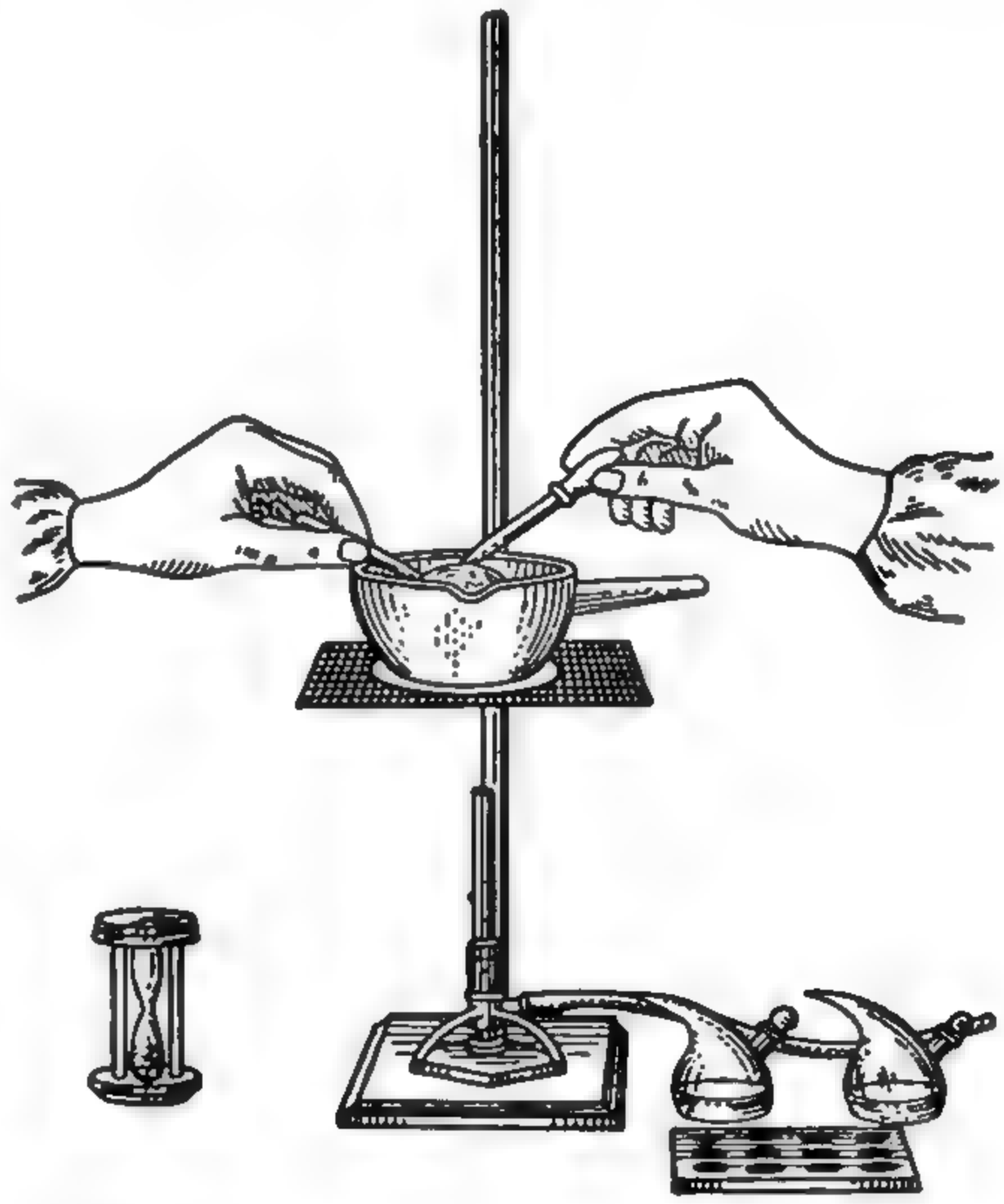


FIG. 273. Ross's method for determining reducing sugars.

Conveniences for making the determination by Soxhlet's method, such as 2-minute sand glass for regulating time of boiling, test plate, dropping bottles for ferrocyanide solution and acetic acid, are shown in Fig. 273.

[12] *J. Anal. Chem.*, 4, 427 (1890).

Violette's Method. A volumetric method of copper reduction, which is used extensively in France, is that of Violette.[13] The proportions of copper sulfate, Rochelle salts, and alkali employed in the Soxhlet method may be used in the Violette determination, or the Violette solution may be taken, which consists of 36.46 g. $CuSO_4 \cdot 5H_2O$, 200 g. Rochelle salts, and 500 g. sodium hydroxide solution of 1.2 sp. gr. made up to 1000 ml.

The Violette solution takes a slightly larger amount of copper sulfate than the Soxhlet solution in order that 1 ml. may correspond to the invert sugar derived from 5 mg. of sucrose or $\frac{3}{3}\frac{6}{4}\frac{0}{2} \times 5 = 5.263$ mg. of invert sugar. The ratio of invert sugar and copper sulfate for the Soxhlet and Violette solutions is accordingly 5 : 34.64 :: 5.263 : 36.46.

The Violette solution is preferred by some chemists for convenience in determining sucrose by the method of inversion and copper reduction.

The end point of the reduction in Violette's method is determined, as in the early process of Barreswil, by the disappearance of blue color from the copper solution. The details of the method are as follows:

Ten milliliters of the mixed copper solution is transferred to a large test tube 20 to 22 mm. in diameter and 22 to 24 cm. long; 5 ml. of distilled water is added, if the solution is rich in reducing sugars, and a few small pieces of pumice stone which have been ignited and then washed in acid and water. The copper solution is then heated to boiling, the grains of pumice stone giving a smooth ebullition and preventing the sudden ejection of liquid from the tube. The sugar solution to be tested, which should have been previously clarified and diluted to about 0.5 to 1.0 per cent invert sugar, is then added from a burette, a few milliliters at a time, the copper solution being boiled for 2 minutes after each addition. As the reduction proceeds, the blue color of the solution becomes more of a reddish violet, due to the diminishing intensity of the blue and the increasing amount of the red cuprous oxide. Towards the end of the reduction it is necessary to hold the tube against a white wall or paper and observe the color of the clear solution, after the red oxide begins to settle. When the final drop of sugar solution discharges the last trace of blue color, the reading of the burette is noted, and the calculation of sugar made as previously described.

A little practice is required in the Violette method in following the disappearance of the blue color. The chemist should standardize his solution against invert sugar, following the same procedure in determining end point as in making an analysis.

[13] Sidersky's "Manuel," p. 95, 1909.

The Violette method is much simpler than the Soxhlet method and for this reason is preferred by many chemists. The Soxhlet method, on the other hand, owing to the more sensitive method of determining the end point of reduction, has a much greater degree of accuracy.

The Violette method has been modified by Spencer[14] so as to include the ferrocyanide test for unreduced copper. Some chemists have also sought to improve the method by employing larger test tubes and using 20 ml. of the mixed copper solution. The possibilities of modification in this direction, of course, are unlimited and do not require special description.

Pavy's Method. Another volumetric process, using the disappearance of blue color as end point, is the method of Pavy,[15] which is based upon the fact that when Fehling's solution is reduced in the presence of ammonia the precipitated cuprous oxide is dissolved as a colorless solution, any unreduced copper being indicated by the characteristic blue color of the cuprammonium compounds. The disturbing influence of the precipitated cuprous oxide upon the color of the solution is thus avoided, and, in the absence of air, the change from blue to colorless at the end point becomes quite sharp.

Pavy's copper solution is prepared as follows: 34.65 g. $CuSO_4 \cdot 5H_2O$, 170 g. Rochelle salts, and 170 g. potassium hydroxide are dissolved in water to 1000 ml. It is preferable, however, as in Soxhlet's method to make up the copper and alkali tartrate solutions separately to 500 ml., and to mix equal quantities of the two just before using; 120 ml. of the mixed copper solution is transferred to a liter flask, 300 ml. of ammonia of specific gravity 0.880 is added and the volume completed to 1000 ml.; 20 ml. of the ammoniacal Fehling's solution is reduced by 0.01 g. glucose.

The reduction is carried out in a flask of about 150-ml. capacity, provided with a two-hole stopper, one opening of which is connected with the tip of the burette containing the sugar solution and the other with a bent glass tube for the escape of air and steam.

Forty milliliters of the ammoniacal copper solution is placed in the flask, and after inserting the stopper the solution is brought to a gentle boil. The sugar solution is then added at the rate of 60 to 100 drops per minute, the discharge being regulated by a screw clamp; the ebullition must be maintained without interruption. When the blue color begins to lighten, the sugar solution is added drop by drop until the last trace of color is just discharged. The end point is made more sensitive by looking through the solution against a white plate.

[14] "Spencer's Handbook for Cane-Sugar Manufacturers," 6th ed., p. 247, 1917.
[15] Pavy's "Physiology of the Carbohydrates," p. 71, London, 1894.

The reduction must be made in the complete absence of air; otherwise the dissolved cuprous oxide will be reoxidized. A precaution sometimes used to prevent the entrance of air through momentary cooling is to use a bent glass exit tube, fitted with a rubber valve, dipping into a beaker of water. Care must also be taken not to prolong the time of reduction, otherwise all the ammonia will be expelled and the cuprous oxide not be dissolved.

In Pavy's method 1 molecule of glucose reduces 6 molecules of cupric oxide instead of 5 molecules as by Fehling's solution. These proportions vary somewhat, however, according to concentration and other conditions of experiment. The solution, therefore, should be standardized against glucose or invert sugar following the exact method employed in analysis.

Pavy's method gives good results when the reduction is carried out with complete exclusion of the air. The extra precautions necessary for making the determination, and the failure of the method to give good results with colored solutions, have prevented the process from becoming generally employed.

Conversion Tables for Volumetric Determination of Sugars. The calculation of reducing sugars by any of the volumetric methods is much simplified by the use of appropriate conversion tables. If a volume of Fehling's solution is taken which always corresponds to a fixed amount of reducing sugar, as, for example, 0.5 g. in Table CV, and the sugar solution for titration is made up so as to contain this same amount of substance (as 0.5 g.) in 1 ml., then the formula for determining reducing sugar becomes

$$R = \frac{0.5 \times 100}{0.5 \times V} = \frac{100}{V}$$

in which R is the percentage of reducing sugar in the substance and V the milliliters of sugar solution necessary for the reduction.

If the substance is very high or very low in reducing sugar, an even fraction or multiple of 0.5 g. may be taken for the amount of substance to be dissolved in 1 ml. Thus for 0.05 g. of substance in 1 ml. $R = 1000/V$, and for 1 g. of substance in 1 ml. $R = 50/V$.

Under the above conditions of analysis a table giving different multiples of the reciprocals of the burette readings will give the corresponding percentages of reducing sugars. The example on p. 753 will illustrate the method for constructing such a table.

The table can, of course, be modified in a great variety of ways to suit individual requirements. A list of reciprocals for assistance in calculating such a table is given in the Appendix (Table 11).

FEHLING'S SOLUTION TAKEN = 0.2 GRAM OF REDUCING SUGAR

Volume of Sugar Solution for Reduction	Reciprocal	Weight of Substance in 1 ml. of Sugar Solution				
		0.40 g.	0.20 g.	0.10 g.	0.04 g.	0.02 g.
V	$\frac{1}{V}$	$\frac{50}{V}$	$\frac{100}{V}$	$\frac{200}{V}$	$\frac{500}{V}$	$\frac{1000}{V}$
ml.		per cent	per cent	per cent	per cent	per cent
20.0	0.05000	2.50	5.00	10.00	25.00	50.00
20.1	0.04975	2.49	4.98	9.95	24.88	49.75
20.2	0.04950	2.48	4.95	9.90	24.75	49.50
20.3	0.04926	2.46	4.93	9.85	24.63	49.26
20.4	0.04902	2.45	4.90	9.80	24.51	49.02
....	.....	....	....	....	.....	.....
30.0	0.03333	1.67	3.33	6.67	16.67	33.33
40.0	0.02500	1.25	2.50	5.00	12.50	25.00
50.0	0.02000	1.00	2.00	4.00	10.00	20.00

It should be mentioned again that strictly correct results are obtained only when the volume of the sugar solution required in the test is practically the same as that used in standardizing the same volume of Fehling's solution. But if, for instance, 20 ml. of sugar solution were used in the standardization, and 50 ml. in the test, then the quantity of Fehling's solution will not be equivalent to 0.2 g. of sugar, but to a slightly different quantity. This point has been well brought out by the authors of the method to be described next.

Lane and Eynon's Volumetric Method.[16] A great advance in volumetric determination of reducing sugars was made by Lane and Eynon when they discovered that methylene blue can be used as an internal indicator of the end point. The experimental precautions necessary in the Pavy method, the difficulty in observing the exact end point in the Violette method, and the time-consuming ferrocyanide spot test in the Soxhlet method are completely avoided, and the precision is very much greater. The methylene blue is added to the reaction mixture, much as starch is used in iodine titrations. Its use is based on the fact that it is reduced and completely decolorized by minute amounts of reducing sugars, but not so long as any cupric salt is present. The reduction is carried out in a flask in which the liquid is kept boiling constantly to prevent reoxidation. Otherwise the method is similar to Soxhlet's, and it is carried out as follows, according to the directions of the Association of Official Agricultural Chemists:[17]

[16] *J. Soc. Chem. Ind.*, **42**, 32T (1923).

[17] "Methods of Analysis, A. O. A. C.," 5th ed., pp. 498–499, 1940.

Standardization and Method of Titration. Pipette accurately 10 or 25 ml. of mixed Soxhlet's reagent or pipette 5 or 12.5 ml. of each of Soxhlet's solutions A and B into a flask of 300–400 ml. capacity. The quantity of copper taken will differ slightly between the two methods of pipetting, and the method used must be carried out consistently during standardization and determination. Prepare a standard solution of the pure sugar of such concentration that more than 15 ml. and less than 50 ml. will be required to reduce all the copper. The titer may be calculated as follows: $\frac{\text{factor}}{\text{mg. sugar in 1 ml.}}$. Add almost the whole of the sugar solution required to effect reduction of all the copper, so that not more than 0.5–1 ml. is required later to complete the titration. Heat the cold mixture to boiling on a wire gauze and maintain in moderate ebullition for 2 minutes, lowering the flame sufficiently to avoid bumping. Without removing the flame add 2–5 drops of 1 per cent aqueous methylene blue solution and complete the titration within a total boiling time of about 3 minutes by small additions of sugar solution to decolorization of the indicator.

Multiply the titer by the number of mg. in 1 ml. of the standard solution to obtain the factor. Compare with the tabulated factor to determine the correction, if any, to be applied to the table. Small deviations from the tabulated factors may arise from variations in individual procedure or composition of reagents. If only approximate results (within 1 per cent) are required the standardization may be omitted, provided that the specifications of the analysis are rigidly observed.

Determination. If the approximate concentration of the sugar in the sample is unknown, proceed by the incremental method of titration. Add to 10 or 25 ml. of Soxhlet's solution 15 ml. of the sugar solution and heat to boiling over a wire gauze. Boil about 15 seconds and add rapidly further quantities of the sugar solution until only the faintest perceptible blue color remains. Then add 2–5 drops of methylene blue and complete the titration by adding the sugar solution dropwise. (The error resulting from this titration will not generally exceed 1 per cent.)

For higher precision repeat the titration, adding almost the whole of the sugar solution required to reduce all the copper, and proceed as directed above. Find the factor corresponding to the titer, and apply the correction previously determined. Estimate as follows:

$$\frac{\text{factor} \times 100}{\text{titer}} = \text{mg. of sugar in 100 ml.}$$

It is important that the flask be not removed from the flame except for a few brief moments when one is in doubt whether the reduction is complete. In this case the flask is held against a white background; if reduction is not complete the edge of the liquid appears bluish. The easiest way to add the sugar solution to the flask is from a burette held in the hand. To prevent heating the burette the tip is bent twice at

right angles. A pinchcock is preferable to a glass cock because the latter is liable to bind.

The Lane and Eynon factors from which the milligrams of sugar are calculated are shown in the Appendix, Tables 12 and 13. It will be noted that the factors vary with the volume of Fehling's solution as well as of sugar solution, for reasons discussed above.

The necessary calculations are greatly simplified, according to Kenny and Fill,[18] by constructing a graph in which the Lane and Eynon titers are plotted directly against milligrams of each sugar in 100 ml. of solution. In this way the high precision of the method is more fully utilized.

Dianol green has been advocated by Mann[19] as an internal indicator in place of methylene blue, for the reason that it is not subject to back oxidation. But Eynon and Lane[20] have pointed out that this is a disadvantage rather than an advantage because it gives a false sense of security. Furthermore, the tinctorial power of methylene blue is so great that the excess sugar solution necessary to reduce it is negligible, while dianol green requires a considerable quantity. The Lane and Eynon factors can therefore not be used when dianol green is used as an indicator.

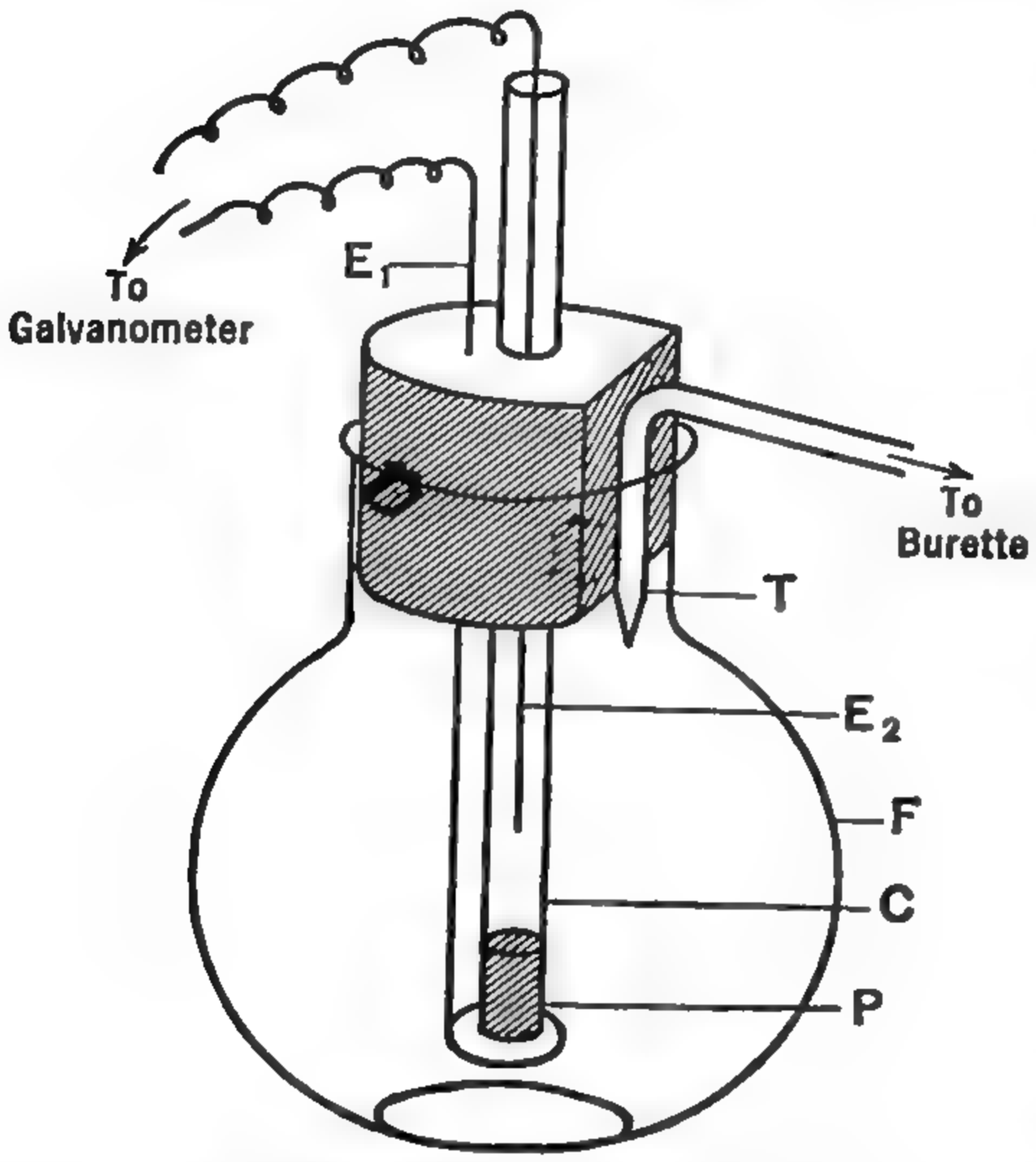
Haddon[21] has proposed to improve the detection of the end point obtained with methylene blue by the addition of 4 ml. of a 10 per cent solution of potassium ferrocyanide to the 10 ml. of Fehling's solution. This produces a sudden disappearance of the blue color and a change to orange. Von Stieglitz[22] has found, however, that the titration values obtained with this modification are entirely different from those found by the original Lane and Eynon procedure.

The principle of Pavy's method (p. 751) has been applied to the Lane and Eynon titration procedure by Ling and Carter,[23] but the complications introduced thereby detract from its usefulness.

In the analysis of very dark molasses it is sometimes difficult to detect the end point with methylene blue as internal indicator, especially in poor light. In such cases it is preferable to determine the end point electrometrically. Such a method has been proposed by Tryller,[24] and recommended by von Stieglitz[25] and others.

[18] *Analyst*, 64, 420 (1939).
[19] *Chemistry & Industry*, 45, 187 (1926).
[20] *Chemistry & Industry*, 45, 545 (1926).
[21] *Rev. agr. Maurice*, No. 59, 131 (1931).
[22] *Proc. Queensland Soc. Sugar Cane Tech.*, 1936, p. 101.
[23] *Analyst*, 55, 730 (1930).
[24] *Intern. Sugar J.*, 34, 353 (1932).
[25] *Proc. Queensland Soc. Sugar Cane Tech.*, 1936, p. 101; 1938, p. 29; 1939, p. 43.

The apparatus, Fig. 274, consists of a round, flat-bottom, wide-mouth flask (Soxhlet extraction flask) of 250-ml. capacity, with a cork stopper one-third of which has been cut away. The sugar solution enters from the burette through tube *T*. The electrodes are of pure copper wire, about 1 mm. thick, and preferably cut from the same coil. The cell *C* in the center is made of Pyrex tubing 8 mm. in internal diameter and 16 cm. long. At its lower end it is filled with a plug, about 8 mm. high, made from a slurry of plaster of Paris of the consistency of thick cream. After the plug has hardened the cell is filled with a mixture of 5 ml. Soxhlet solution B (alkaline tartrate), 5 ml. of a solution containing 39.415 g. anhydrous sodium sulfate per liter, and 20 ml. of distilled water. The cell must then be allowed to stand for several hours, preferably overnight, to saturate the plug. A number of cells may be prepared at one time, partially filled with sodium sulfate solution, and kept in a stoppered jar containing the same solution, until required.



(*Reproduced with permission from Proc. Queensland Soc. Sugar Cane Tech., 1938, p. 30.*)

FIG. 274. Von Stieglitz's apparatus for electrometric determination of the end point.

One electrode is immersed in the cell liquid, the other in the test liquid, and the two are connected through a sensitive galvanometer with a central 0 point and a tapping key. A sensitivity of 2 microamperes per division is satisfactory.

The determination is carried out in the usual manner with 10 ml. of mixed Fehling's solution, up to the point where the methylene blue would be added. The tapping key is pressed down, and the needle will be found to swing to one side. As the sugar solution is added drop by drop, the swings of the needle become smaller and smaller, and when the end point is reached the needle swings to the other side. Toward the end it is well to wait about 5 seconds between additions of sugar solution, because there is a slight lag in obtaining equilibrium. Duplicate tests usually agree within about 0.05 ml., the best results being obtained when the titration values are between 15 and 30 ml. If the

titration values lie between 30 and 50 ml., the solution in the reference cell should be made up with 40 instead of 20 ml. of distilled water in order that the concentration approximate more closely that of the liquid in the flask. After each titration the exposed end of the plaster plug should be cleaned with a stiff brush to remove any deposit which may have formed; the tube is then rinsed with distilled water. The copper electrodes should also be kept clean by polishing them occasionally with fine emery paper.

The cell liquid should be renewed after every 4 determinations, and the plugs replaced by new ones after about 40 determinations.

In the analysis of solutions of known sugar content von Stieglitz observed excellent checks with the results by the usual Lane and Eynon procedure. The method has been officially adopted in Queensland.

A method in which the end point of the reduction is determined by measuring the oxidation-reduction potential with an electrometer has been described by Cantor and Leuck[26] but has not been subjected yet to confirmatory work.

Main's " Pot " Method.[27] Further refinements have been added to the Lane and Eynon method by Main, who calls attention to the fact that the time of heating and the rate of ebullition are difficult to standardize and that for this reason variable results may be obtained. To obviate this he adopts the principle previously employed by Reischauer and Kruis (see p. 760) of using the temperature of the boiling-water bath for the reduction. In order to prevent reoxidation of the copper he uses test tubes provided with floats. Three or more tubes are prepared, each containing the same amount of copper reagent but increasing quantities of sugar solution. At the end of the reaction the methylene blue in some of the tubes retains its color, while in at least one it should be decolorized. By repeating the test, gradually narrowing down the increments in the volume of sugar solution, the volume necessary exactly to reduce the copper solution can be determined very accurately.

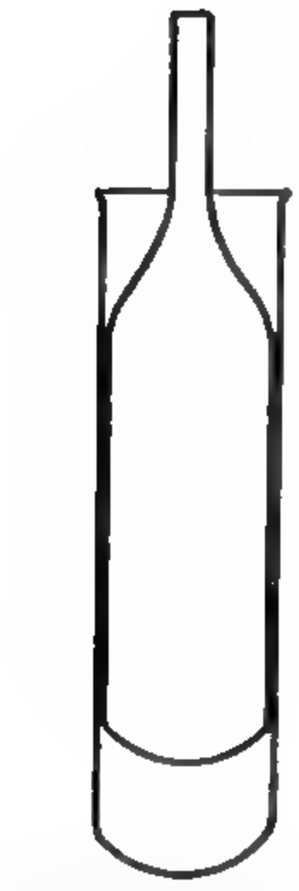
Main describes the details of his method as follows:

The tubes should be selected of nearly the same size and weight, as of course a thin glass allows the contained solution to heat up more rapidly than thick. The tubes, made of resistance glass, are 150 mm. long, 38 mm. internal diameter, and weigh between 50 and 55 g. The floats are similar test tubes, which make a sliding fit in the others. It is convenient to have them drawn out to a

[26] Paper presented at the Boston meeting of the American Chemical Society, September 11 to 15, 1939.

[27] *Intern. Sugar J.*, 34, 213, 460 (1932).

taper some 100 mm. from the closed end, making a total length of about 170 mm. (Fig. 275). The water bath is an ordinary oval iron kitchen pot, tinned inside, the capacity of which is 3 gallons. An overflow is fitted near the upper edge of the boiler, and through a sight feed hot water is added continuously to replace the loss by evaporation (Fig. 276). The temperature of



(Reproduced with permission from Intern. Sugar J., 34, 214.)

FIG. 275. Reduction tube with float for Main's pot method.

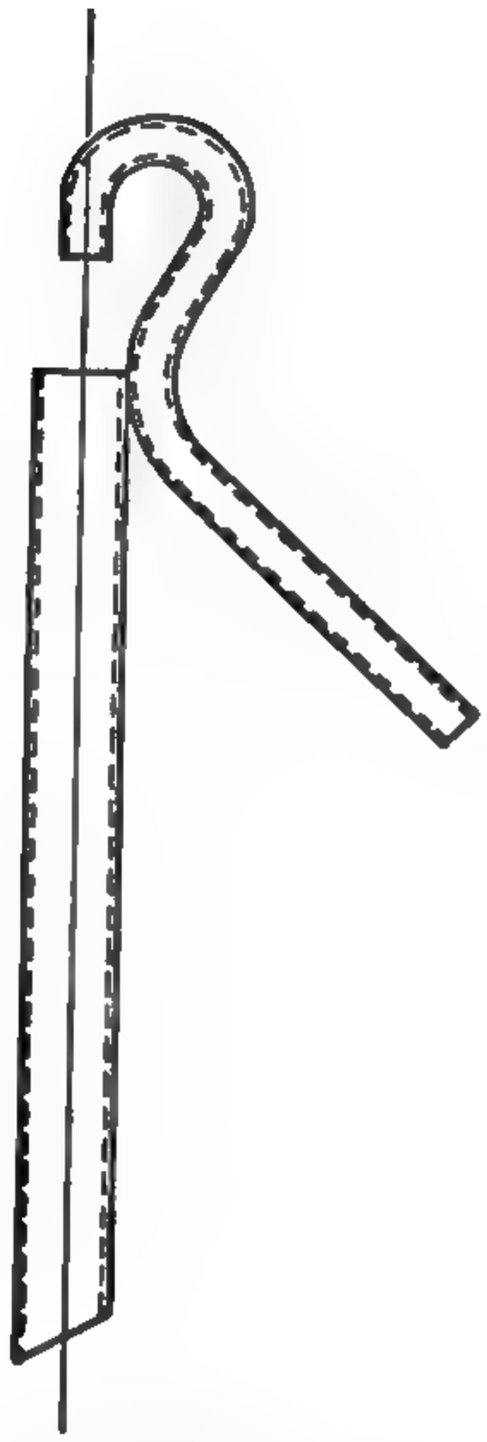


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FIG. 276. Sight feed for water bath in Main's pot method.

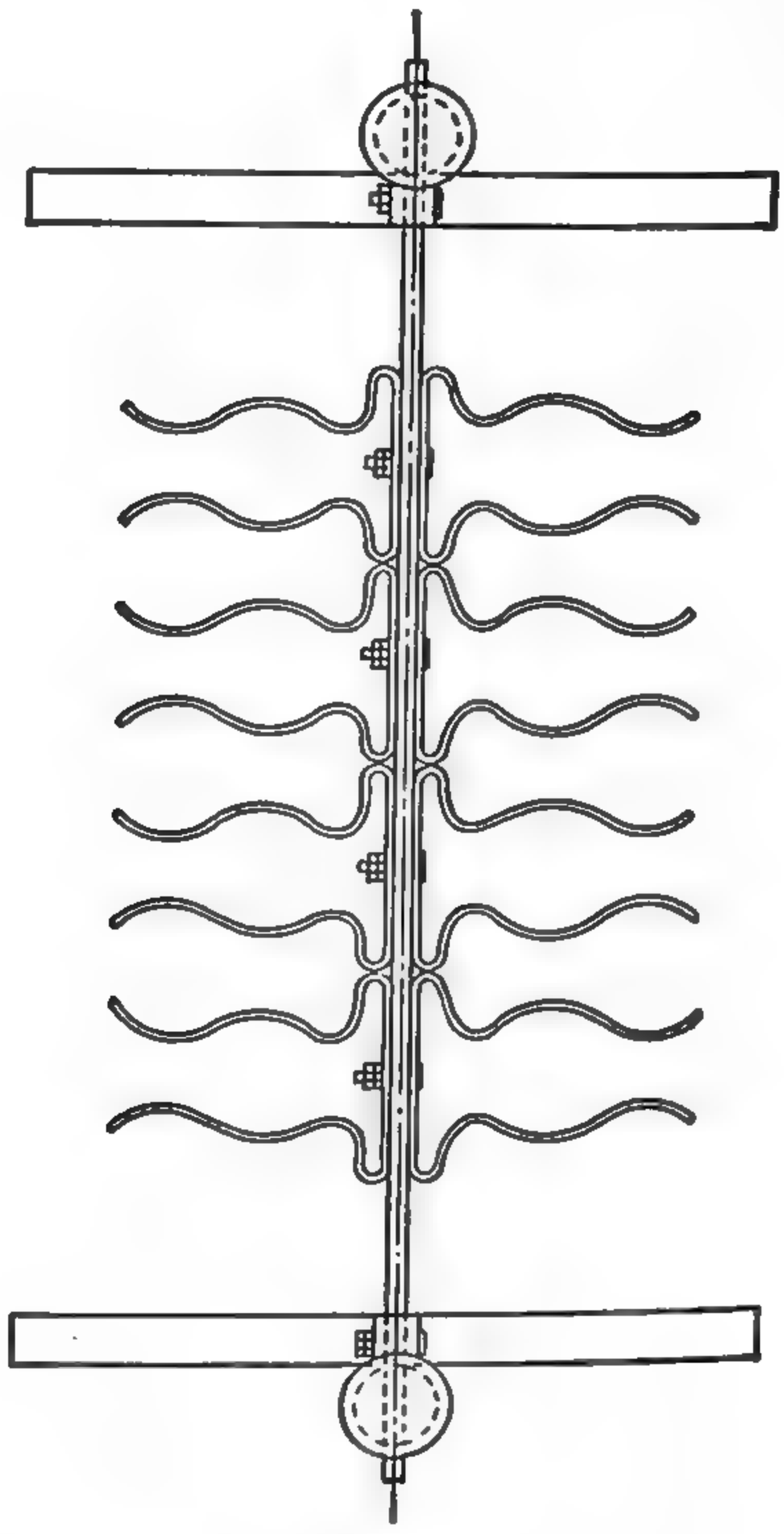

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FIG. 277. Carrier for reduction tubes in Main's pot method.

the water must be maintained at the boiling point, for which a large ring gas burner is necessary. While in the water bath, the tubes are supported by clips in a carrier, a diagram of which is shown (Fig. 277).

The Soxhlet solution and the methylene blue solution are the same as in the Lane and Eynon method. Standard invert sugar solution is prepared as follows:

Accurately weigh 9.5 g. of pure sucrose, dissolve in about 80 ml. of distilled water, add 5.3 ml. of hydrochloric acid, sp. gr. 1.16, and dilute with water to approximately 100 ml. Allow this solution to stand at 22–25° C. for 3 or 4 days and then dilute to 1 liter with distilled water. The solution now contains 0.01 g. invert sugar per ml.

Put into three tubes the following solutions in the order stated: (1) Ten milliliters Soxhlet solution in each tube. (2) Diluted invert-sugar solution, 24.5, 25, and 25.5 ml. into the three tubes respectively; this dilute solution is prepared by taking 50 ml. of standard invert-sugar solution, neutralizing with caustic soda solution (approximately 2.5 ml. normal sodium hydroxide will be required), and completing the volume to 250 ml. with distilled water. This solution now contains 0.002 g. invert sugar per ml. (3) Two drops of the 1 per cent methylene blue solution in each tube.

Mix the contents of each tube by gentle rotation and insert the floats so that they rest on the liquid, care being taken not to entrap any air bubbles. The floats may be pushed well into the liquid to force out air bubbles; on releasing the pressure the floats will, of course, rise to the surface. Place the tubes in the carrier from left to right and transfer to the pot of briskly boiling water for exactly 5 minutes. Then remove and inspect.

The tube containing 25.5 ml. invert-sugar solution should be quite red, indicating that complete reduction has taken place; the middle one should also be reduced, but the left-hand one, containing only 24.5 ml. invert-sugar solution, should still be quite blue. The exact amount of invert-sugar solution required for the reduction of the 10 ml. Soxhlet solution is therefore between 24.5 and 25 ml. — midway is taken as an approximation. Therefore 10 ml. Soxhlet solution is equivalent to 24.75×0.002 g. = 0.0495 g. of invert sugar.

Closer approximation to the true result may be attained by lessening the intervals between the volumes in the tubes. The mean between the last blue and the first red is always taken as the true result unless the blue color is actually seen to fade in a tube on removing it from the pot at the end of the 5 minutes. In that case, the actual volume in that tube is taken as the correct figure.

It is important to measure the 5 minutes with precision. An interval timer clock which rings a bell after the expiration of 5 minutes is a very simple and useful adjunct; but, of course, a stop watch may be employed.

The only reducing sugar for which Main has constructed a table is invert sugar (see Appendix, Tables 14 and 15).

Main[28] later observed that the standard invert-sugar solution may vary slightly in its reducing power, and he therefore recommends pure dextrose for standardization.

Other volumetric methods for the determination of reducing sugars in general, with electrometric end-point indication, have been described

[28] *Intern. Sugar J.*, 35, 43 (1933).

by Daggett, Campbell, and Whitman,[29] and by Niederl and Müller.[30] But both these methods require more complicated apparatus than the simple procedure of von Stieglitz as applied to the Lane and Eynon method (p. 756) and therefore offer no practical advantages.

Reischauer and Kruis's Method. In the methods previously described a constant volume of Fehling's solution was taken and the amount of sugar solution necessary to complete the reduction was noted. In a process first proposed by Lippmann[31] and elaborated by Reischauer and Kruis[32] the opposite procedure is followed. A constant volume of sugar solution is taken and the amount of Fehling's solution necessary to oxidize the reducing sugar is determined.

In the Reischauer-Kruis method the sugar solution is made up so as not to contain over 0.58 g. glucose in 100 ml. Six numbered test tubes holding from 20 to 30 ml. are taken and 5 ml. of the sugar solution measured into each; 1, 2, 3, 4, 5, and 6 ml. respectively of Fehling's solution are then added to the different tubes, which are afterwards shaken and immersed in boiling water for 20 minutes. At the end of this time the tubes are examined and the two tubes noted in which reduction is just completed and in which the least amount of unreduced copper is left. The limits between which the true copper equivalent lies having been noted, the volume of Fehling's solution is varied within this interval until the exact amount necessary for oxidizing all the reducing sugar is found.

The pipettes employed for this method are graduated in their lower part from 1 ml. to 5 ml. and in the stem contain an extra 1 ml. graduated into hundredths. With three trials and employment of the ferrocyanide test, the volume of Fehling's solution can be determined to 0.01 ml. The following example illustrates the application of the method.

First Trial	Second Trial	Third Trial
1 ml. Cu all reduced	4.15 ml. Cu all reduced	4.32 ml. Cu all reduced
2 ml. Cu all reduced	4.30 ml. Cu all reduced	4.34 ml. Cu all reduced
3 ml. Cu all reduced	4.45 ml. Cu in solution	4.36 ml. Cu all reduced
4 ml. Cu all reduced	4.60 ml. Cu in solution	4.38 ml. Cu in solution
5 ml. Cu in solution	4.75 ml. Cu in solution	4.40 ml. Cu in solution
6 ml. Cu in solution	4.90 ml. Cu in solution	4.42 ml. Cu in solution

[29] *J. Am. Chem. Soc.*, **45**, 1043 (1923).
[30] *J. Am. Chem. Soc.*, **51**, 1356 (1929).
[31] *Oesterr. ungar. Z. Zuckerind. Landw.*, **7**, 256 (1878).
[32] *Oesterr. ungar. Z. Zuckerind. Landw.*, **12**, 254 (1883).

The quantity of Fehling's solution which exactly oxidizes the reducing sugar in the 5 ml. of solution may, therefore, be placed at 4.37 ml.

The amount of glucose corresponding to each 0.01 ml. between 1 ml. and 6 ml. of Fehling's solution is found from a table calculated by Kruis.

The methods of Main and of Reischauer-Kruis possess certain advantages over the methods previously described in point of exactness; the error due to variation in reducing power with changes in concentration is avoided, the amount of reducing sugar corresponding to different volumes of Fehling's solution, or vice versa, being definitely known for the conditions of the experiment. The large amount of labor and time necessary for completing a determination, however, is a serious obstacle against the general use of these methods.

Methods Employing an Excess of Copper Reagent and Filtration of the Cuprous Oxide

In the methods of this class an excess of copper is present in the Fehling's solution at the end of reduction. The precipitated cuprous oxide after a fixed period of heating is filtered off, and the amount of copper is determined by any of the numerous gravimetric or volumetric processes. The weight of reducing sugar corresponding to a definite weight of precipitated copper is then determined by means of formulas or tables which have been calculated from results obtained upon known amounts of pure sugar under similar conditions of experiment.

Variability in Reducing Power of Monosaccharides. Soxhlet[33] showed that when a solution of glucose acted upon Fehling's solution the first portion added reduced most strongly and the succeeding portions gradually less so. This variability in reducing power is found to be different, however, for the monosaccharides, glucose, fructose, invert sugar, galactose, etc., than for the disaccharides, lactose and maltose.

As examples of the variability in reducing power of monosaccharides the following results are given. The values, which were calculated from Bertrand's sugar tables, represent the milligrams of copper reduced by each succeeding 10-mg. portion of added sugar (Table CVI).

[33] *J. prakt. Chem.*, [2], **21**, 227 (1880).

TABLE CVI

Variability in Reducing Power of Monosaccharides

Number of Series		Invert Sugar. Milligrams Copper	Glucose. Milligrams Copper	Galactose. Milligrams Copper
First	10 mg. of sugar reduce.....	20.6	20.4	19.3
Second	10 mg. of sugar reduce.....	19.8	19.7	18.6
Third	10 mg. of sugar reduce.....	18.9	19.0	18.3
Fourth	10 mg. of sugar reduce.....	18.4	18.4	17.7
Fifth	10 mg. of sugar reduce.....	17.7	17.9	17.3
Sixth	10 mg. of sugar reduce.....	17.2	17.4	16.9
Seventh	10 mg. of sugar reduce.....	16.6	17.0	16.7
Eighth	10 mg. of sugar reduce.....	16.1	16.3	16.3
Ninth	10 mg. of sugar reduce.....	15.8	15.9	16.3
Tenth	10 mg. of sugar reduce.....	15.4	15.8	16.0

It is seen that each succeeding 10 mg. of added glucose undergoes a loss in reducing power of about 3 per cent.

Law of Reducing Action. The reducing action of a monosaccharide upon Fehling's solution may be expressed in general terms as follows:

If for the first minute quantity s of a given sugar a definite amount c of copper is reduced, then for any multiple n of s the weight of copper would be nc, if the same amount of copper in the Fehling's solution were always maintained. The latter condition, however, is never realized in practice, and with the continuous removal of copper from solution the value nc becomes $nc - (n-1+n-2+n-3+\cdots n-n)k$. When working with weighable quantities of sugar, this expression should be modified to $c+(n-1)d-(n-2+n-3+\cdots n-n)k$, in which d is the difference between the weights of copper for the first two members of the series s and $2s$. The values of d and of the constant k are easily determined empirically, and these being known it is possible to construct tables for any of the reducing sugars.

As an example of this method of calculation the following values are taken from the experimental work of Allihn:[34]

No. of Series (n)

1.....10 mg. of glucose reduce 18.0 mg. copper
2.....20 mg. of glucose reduce 38.2 mg. copper
25.....250 mg. of glucose reduce 463.0 mg. copper

$$18.0 = c$$
$$38.2 - 18.0 = 20.2 = d$$

[34] *J. prakt. Chem.*, [2], **22**, 46 (1880).

Substituting the above values for c and d in the equation for $n = 25$,

$$18 + (25 - 1)\,20.2 - (25 - 2 + 25 - 3 \ldots)\,k = 463.0$$

whence

$$k = 0.14$$

The equation $18 + (n - 1)\,20.2 - (n - 2 + n - 3 + \cdots n - n)\,0.14$ will give the milligrams of copper reduced by any multiple n of 10 mg. of glucose under the conditions of Allihn's experiments.

Suppose it to be required to find the milligrams of copper reduced by 100 mg. of glucose.

$$18 + (10 - 1)\,20.2 - (10 - 2 + 10 - 3 \ldots)\,0.14 = 194.8 \text{ mg. Cu}$$

Allihn obtained by actual experiment 195 mg. of copper by the reducing action of 100 mg. of glucose.

Calculation of Reduction Tables. The calculation of tables for the copper-reducing power of different sugars is usually made by the method of least squares, according to the general formula:

$$y = A + Bx + Cx^2$$

in which x is the milligrams of copper reduced by y milligrams of sugar and A, B, and C constants. The values of x having been determined by experiment for 10 or more values of y, the calculation of A, B, and C is made in the same manner as described on p. 266.

As an example of the method of least squares the work of Allihn is again quoted. Allihn found that different amounts of glucose under constant conditions of experiment reduced the following amounts of copper.

Mg. of glucose (y) .	10.0	20.0	25.0	50.0	100.0	125.0	150.0	175.0	200.0	225.0	250.0
Mg. of copper (x) . .	18.0	38.2	47.5	99.0	195.0	242.5	287.7	333.0	377.7	421.2	463.0

Substitution of the above values for x and y in the formula $y = A + Bx + Cx^2$ gives the general equation

$$y = -2.5647 + 2.0522\,x - 0.0007576\,x^2$$

by means of which Allihn constructed his table giving the milligrams of glucose corresponding to any weight of reduced copper between 10 mg. and 463 mg.

The values found experimentally by Allihn show that, beginning at 0 glucose, each additional 50 mg. of it reduce 99.0, 96.0, 92.7, 90.0, and 85.3 mg. copper, as postulated by the law of reducing action. Between 0 and 50 mg. of glucose the quantity of copper reduced by each milligram of glucose varies somewhat. For instance, the first 10 mg. gave 18.0 mg. copper, but the second 10 mg. gave 20.2. This variable reducing power for small amounts of sugar, when determined by gravi-

metric methods, is probably due to a colloidal condition of the precipitate which is partly lost by running through the filter.

In a study of the Munson and Walker method (see p. 801), Hammond[35] found that either the parabola or the hyperbola expresses the experimental data very well up to a certain limit of sugar concentration. But as the copper solution becomes nearly exhausted the quantity of copper reduced by equal quantities of sugar decreases more rapidly, and the total amount of copper reduced reaches a limiting value asymptotically. The equation of the entire curve is that of a hyperbola with a correcting term:

$$x = \frac{c + d(y_1 - y)^{-1}}{b - y} - a$$

where x is milligrams sugar, y milligrams copper, and y_1 the limiting value of copper (in the Munson and Walker method 440.9 mg.); a, b, c, and d are constants whose value varies with each individual sugar. The correcting term is $d(y_1 - y)$.

Variability in Reducing Power of Disaccharides. The variability in reducing power of maltose and lactose is different from that noted for the monosaccharides. According to the amount of free alkali, time of boiling and other conditions, succeeding portions of maltose and lactose, while usually showing a slight loss, may show either no change at all, or even a slight gain in reducing power over preceding portions of the same sugar. This peculiarity of maltose and lactose is explained by a slight hydrolysis of the sugar into monosaccharides of higher reducing power. A slight inversion of this kind takes place with sucrose, which is strictly speaking a non-reducing sugar, and it no doubt occurs to a greater or less extent with all higher saccharides upon boiling with Fehling's solution.

As an illustration of the reducing power of successive portions of maltose, the results in Table CVII are taken from the tables of Wein and of Munson and Walker.

It is seen that in both series of experiments there is at first a marked decrease and then a slight increase in the reducing power of the successive portions of added sugar. Changes of a similar nature are noted in some of the tables for lactose (see Appendix, Table 12).

The reducing power of the disaccharides upon Fehling's solution is much more subject to change with difference in conditions than the monosaccharides. Kjeldahl,[36] for example, found that increasing the amount of alkali in Fehling's solution caused the reducing power of

[35] *J. Research Nat. Bur. Standards*, **24**, 579 (1940).
[36] *Neue Z. Rübenzuckerind.*, **37**, 13, 23 (1887).

TABLE CVII

Variability in Reducing Power of Maltose

Number of Series		Wein	Munson and Walker
		mg. Cu	mg. Cu
First	30 mg. of maltose reduce	35.4	35.9
Second	30 mg. of maltose reduce	34.5	33.6
Third	30 mg. of maltose reduce	34.0	33.5
Fourth	30 mg. of maltose reduce	33.4	33.8
Fifth	30 mg. of maltose reduce	33.4	33.6
Sixth	30 mg. of maltose reduce	33.8	33.7
Seventh	30 mg. of maltose reduce	33.5	33.6

maltose and lactose to gain with ten times the rate of increase noted for glucose. The same effect is also produced by prolonging the time of boiling. This greater sensibility of the disaccharides to disturbing influences during reduction involves a greater experimental error in the determination when the details of the method are not carefully followed.

Methods and tables for estimating different sugars from the amount of copper reduced from Fehling's solution have been devised by Soxhlet; Allihn; Wein; Meissl; Herzfeld; Lehmann; Kjeldahl; Pflüger; Ost; Hönig and Jesser; Brown, Morris, and Millar; Bertrand; Defren; Munson and Walker; Kendall; and many others. It is impossible to describe all these processes, and only a few of the more typical methods will be selected. The procedure of Allihn,[37] which is one of the classical copper-reduction methods, illustrates well the various principles involved and will be described first in some detail.

Allihn's Method for the Determination of Glucose. The following details of Allihn's method with the description of several processes for determining the amount of reduced copper are taken from the Methods of Analysis of the Association of Official Agricultural Chemists.[38]

PREPARATION OF REAGENTS

Copper Sulfate Solution. Dissolve 34.639 g. of $CuSO_4 \cdot 5\,H_2O$ in water and dilute to 500 ml.

Alkaline Tartrate Solution. Dissolve 173 g. of Rochelle salts and 125 g. of potassium hydroxide in water and dilute to 500 ml.

[37] *J. prakt. Chem.*, [2], 22, 46 (1880).

[38] " Methods of Analysis, A. O. A. C.," 5th ed., p. 504, 1940.

DESCRIPTION OF METHOD

Place 30 ml. of the copper solution, 30 ml. of the alkaline tartrate solution and 60 ml. of water in a beaker and heat to boiling. Add 25 ml. of the solution of the material to be examined, which must be so prepared as not to contain more than 0.250 g. of glucose, and boil for exactly 2 minutes, keeping the beaker covered. Filter immediately through asbestos without diluting, and obtain the weight of copper by one of the methods described in the following section. The corresponding weight of glucose is found from Allihn's table (Appendix, Table 16).

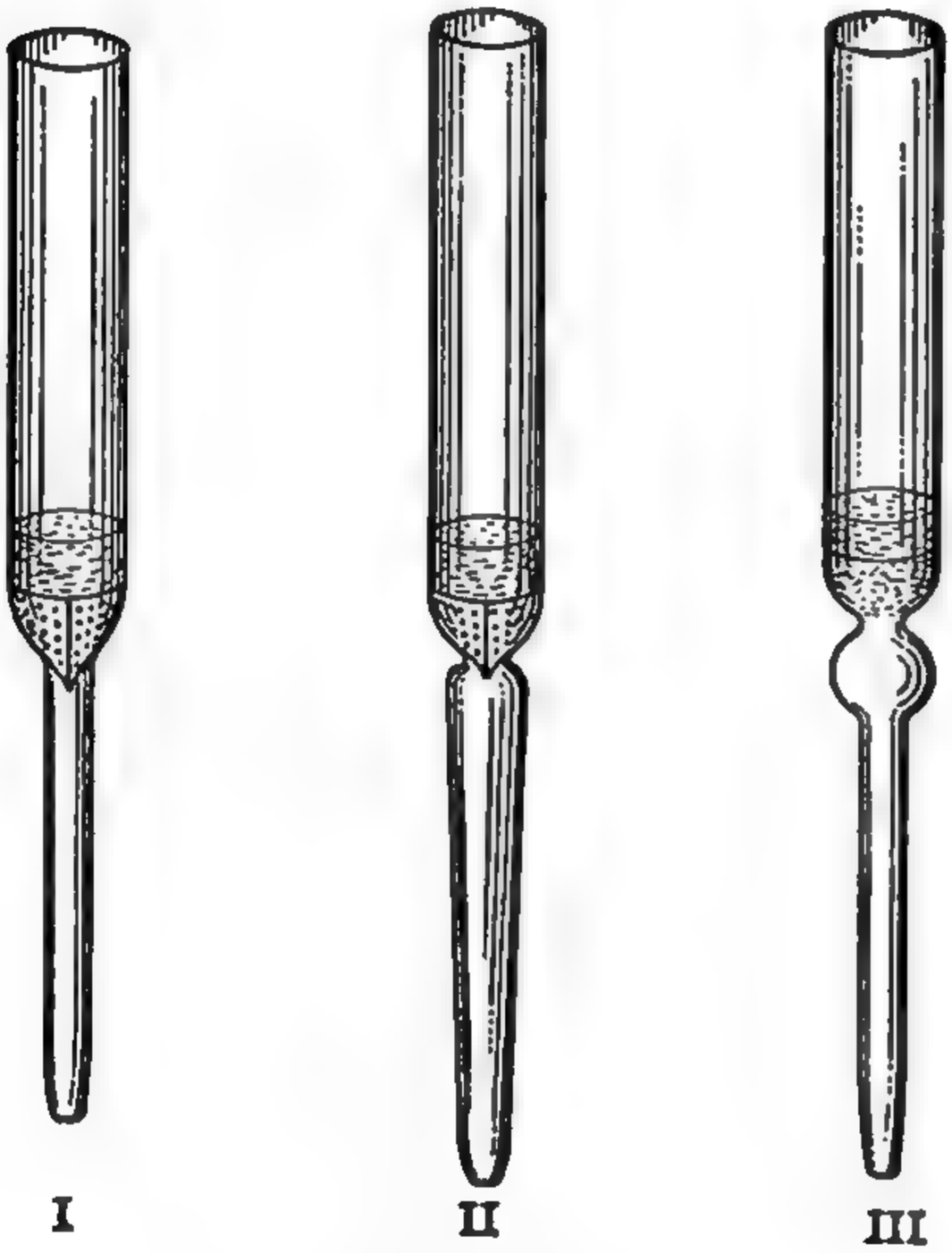


FIG. 278. Forms of tubes for filtering cuprous oxide.

Gooch crucibles of 25- to 35-ml. capacity, with asbestos pads, are most widely used for filtering off the precipitate, having replaced the glass filtering tubes (Fig. 278) which are now employed only in those rare cases where reduction in hydrogen is practiced. If the precipitate is not to be weighed but redissolved for further treatment, an ordinary glass funnel with perforated filter plate and asbestos pad may be substituted for the Gooch crucible.

Preparation of Asbestos. Digest the asbestos, which should be of the amphibole variety, with hydrochloric acid (1 + 3) for 2–3 days. Wash free from acid, digest for a similar period with 10 per cent sodium hydroxide solution, and then treat for a few hours with hot alkaline tartrate solution (old alkaline tartrate solutions that have stood for some time may be used for this purpose) of the strength used in sugar determinations. Wash the asbestos free from alkali; digest for several hours with nitric acid (1 + 3); and, after washing free from acid, shake with water into a fine pulp. In preparing the Gooch crucible, make a film of asbestos $\frac{1}{4}$ inch thick and wash thoroughly with water to remove fine particles of asbestos. If the precipitated cuprous oxide is to be weighed as such, wash the crucible with 10 ml. of alcohol, then with 10 ml. of ether; dry for 30 minutes at 100°; cool in a desiccator; and weigh. It is best to dissolve the copper with nitric acid each time after weighing and use the same felts over and over again, as they improve with use.

A convenient method of filtering cuprous oxide by means of suction is shown in Fig. 279. A continuous filtration should be maintained and all the precipitate should be transferred to the crucible or tube before the liquid above the asbestos is allowed to run completely through. Too rapid or too irregular filtration may cause particles of

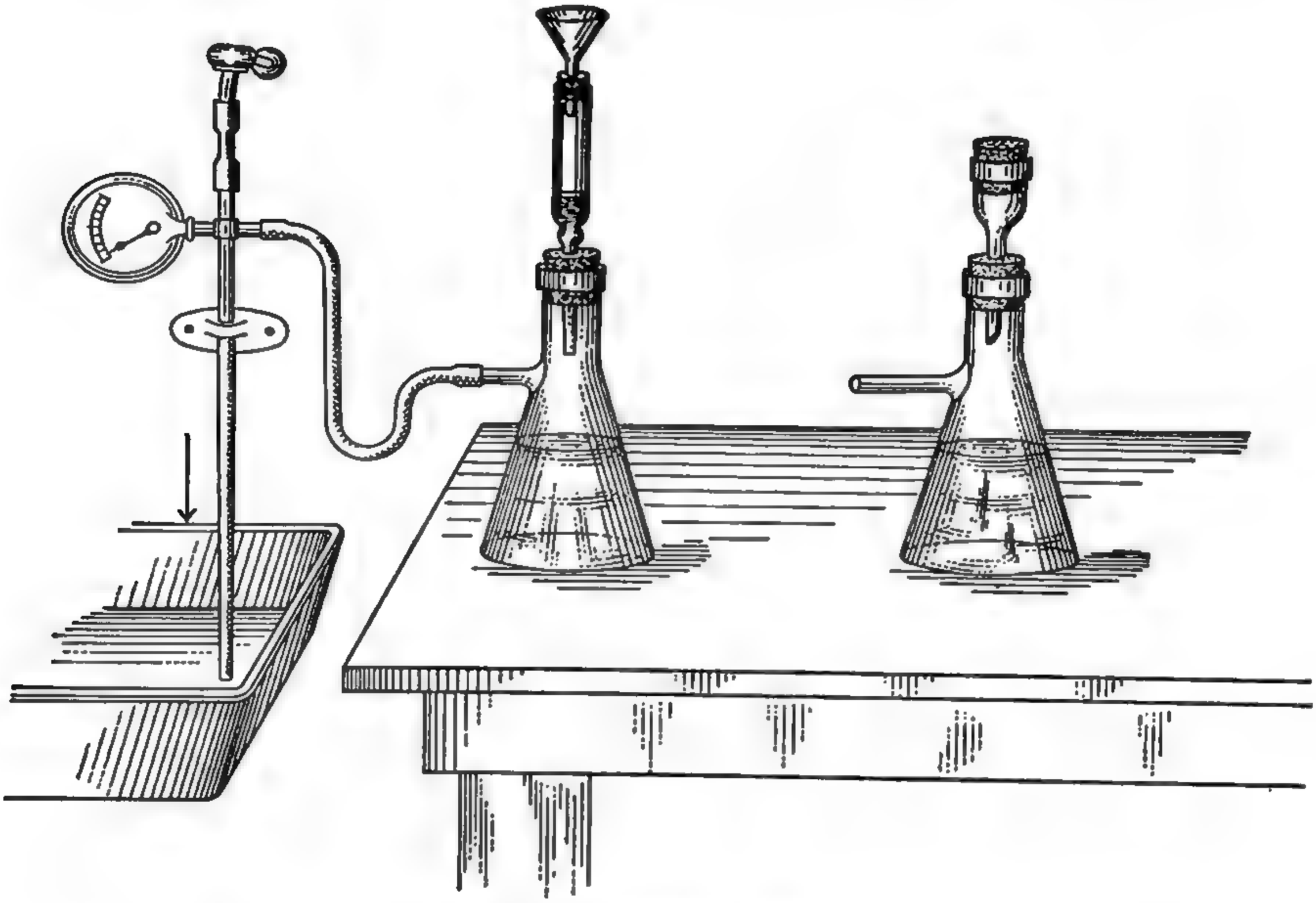


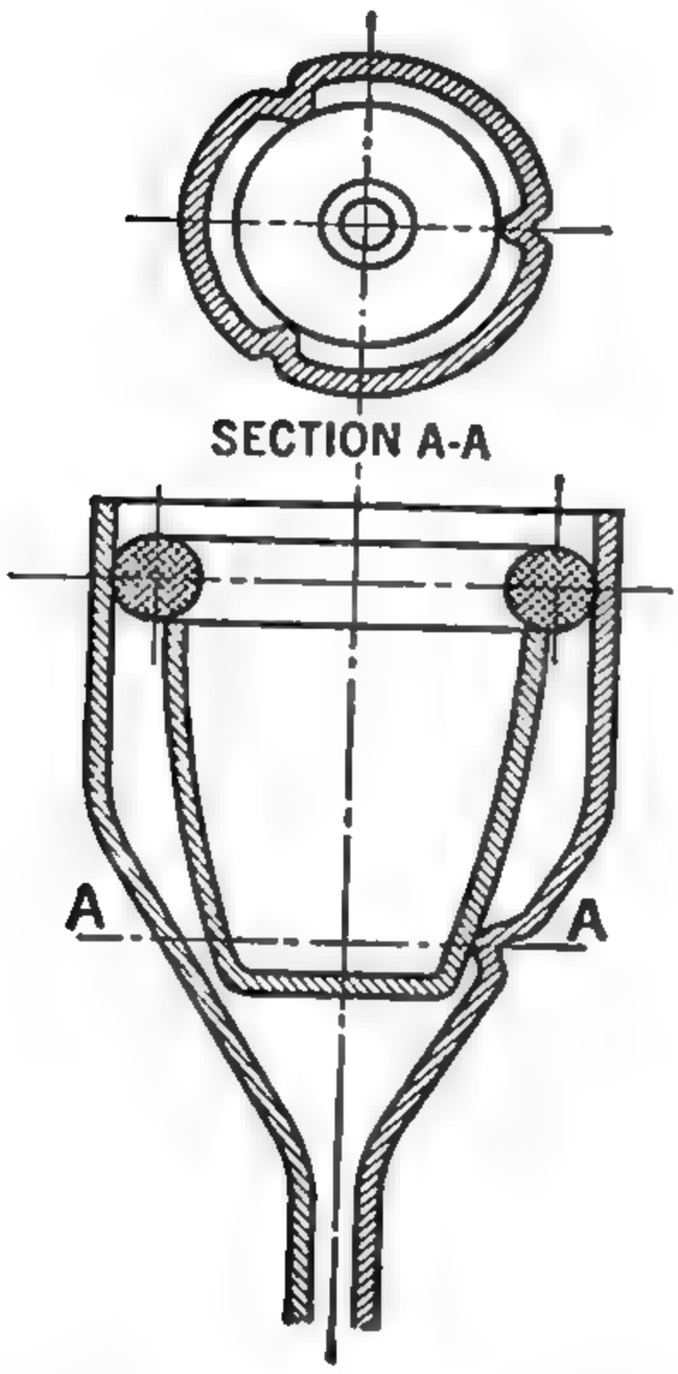
FIG. 279. Showing methods of filtering cuprous oxide with filter tube or Gooch crucible.

cuprous oxide to run through the asbestos. A fine jet of water will usually bring all the cuprous oxide into the crucible or filter tube; should any of the precipitate remain adhering to the beaker, a feather or a rubber-tipped rod will assist the removal.

Some chemists prefer alundum crucibles for the filtration because they do not require the preparation of asbestos pads. Since these crucibles are porous throughout they must be placed in special holders, such as Spencer's (Fig. 280), which permit thorough washing. Meade[39] recommends a crucible of porosity RA 360, and 25-ml. capacity. When the crucibles are new they should be digested first with dilute nitric acid and then with hot mixed Soxhlet solution, after which they are washed

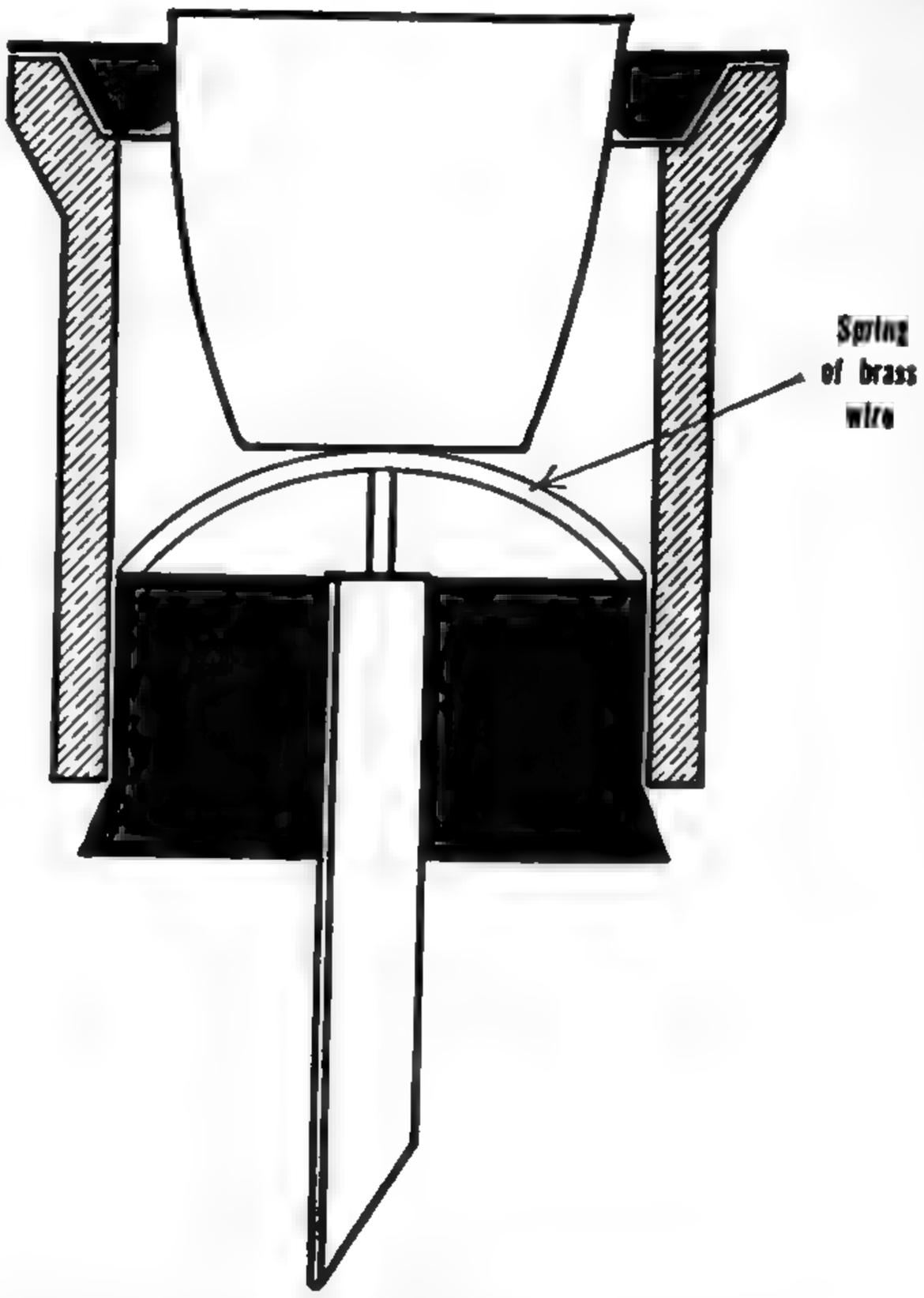
[39] Meade, "Spencer's Handbook," 7th ed., p. 242, 1929.

with hot water. To clean the crucibles thoroughly between tests, a reverse washing apparatus (Fig. 281) is used which serves also in the



(Reproduced with permission from Spencer-Meade, "Handbook for Cane-Sugar Manufacturers," p. 242.)

FIG. 280. Spencer crucible holder.



(Reproduced with permission from Spencer-Meade, "Handbook for Cane-Sugar Manufacturers," p. 243.)

FIG. 281. Washing apparatus for cleaning alundum crucibles.

place of the Spencer holder. The brass spring is removed; the crucible is placed upside down on the rubber stopper and washed from the outside with hot water.

GRAVIMETRIC METHODS FOR DETERMINING COPPER IN FILTERED PRECIPITATE

Direct Weighing of the Cuprous Oxide.[40] Collect the precipitated Cu_2O on the asbestos mat and wash thoroughly with hot water, then with 10 ml. of alcohol, and finally with 10 ml. of ether. Dry the precipitate for 30 minutes in a water oven at the temperature of boiling water, cool, and weigh. Calculate the weight of metallic copper, using the factor 0.8882.

The weight of the particular reducing sugar being determined, corresponding to the weight of the copper, is found from the tables for the various

[40] "Methods of Analysis, A. O. A. C.," 5th ed., p. 500, 1940.

methods (Appendix). The sugar tables of Munson and Walker express results also directly in terms of cuprous oxide.

Weighing as Cupric Oxide. The precipitate is filtered through the Gooch crucible as above, and washed with hot water. Alcohol and ether may be omitted. The crucible is heated first at a little above 100° C. to dry the precipitate, and then placed for 15–30 minutes in an electric muffle furnace to oxidize the cuprous to cupric oxide, at red heat. If the crucible is heated over a gas flame care must be taken that the oxide is not exposed to the action of the reducing part of the flame during ignition; in this case it is also advisable to use Gooch crucibles with open bottoms and separated perforated disks (Caldwell's crucible, Fig. 282), because the one-piece Gooch crucible is liable to crack when heated quickly to high temperatures.

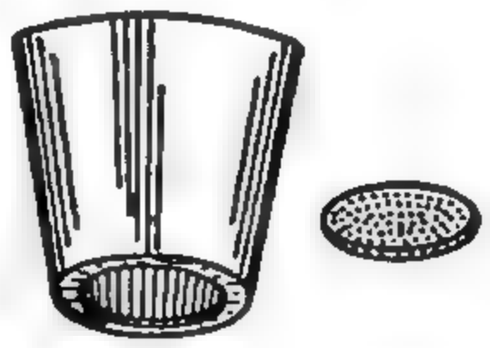


FIG. 282. Caldwell's crucible with detachable bottom.

Finely divided cupric oxide is hygroscopic and, after cooling in a desiccator, should be weighed as quickly as possible. The weight of cupric oxide multiplied by the factor 0.7989 gives the weight of metallic copper. Several sugar tables, as Kjeldahl's and Defren's, express results in terms of cupric oxide, thus avoiding the labor of calculation, when this method of determining copper is used.

The method of estimating copper from the weight of cupric oxide is one of the most accurate of the gravimetric methods. It must be borne in mind, however, that under certain conditions the cupric oxide may be contaminated with mineral matter.

Weighing as Copper after Reduction with Hydrogen.[41] Deposit an asbestos film on a perforated platinum disk, or cone, contained in a hard glass filtering tube, wash free from loose fibers, dry, and weigh. Through this tube, previously mentioned, filter the cuprous oxide immediately, using suction. Transfer the cuprous oxide to the tube through a removable funnel and wash through with hot water, alcohol, and ether successively. After drying connect the tube with a supply of dry hydrogen, heat gently until the cuprous oxide is completely reduced to metallic copper, cool in a current of dry hydrogen, and weigh. If preferred, a Gooch crucible may be used for the filtration.

Several forms of tubes in which the cuprous oxide is filtered on asbestos and reduced are shown in Fig. 278. Glass wool is sometimes used in place of a perforated platinum cone for holding the asbestos, but makes a less resistant support (Fig. 278 III).

[41] "Methods of Analysis, A. O. A. C.," 3d ed., p. 381, 1930

The reduction of the cuprous oxide to copper by means of hydrogen is shown in Fig. 283. All air must be expelled from the tube before

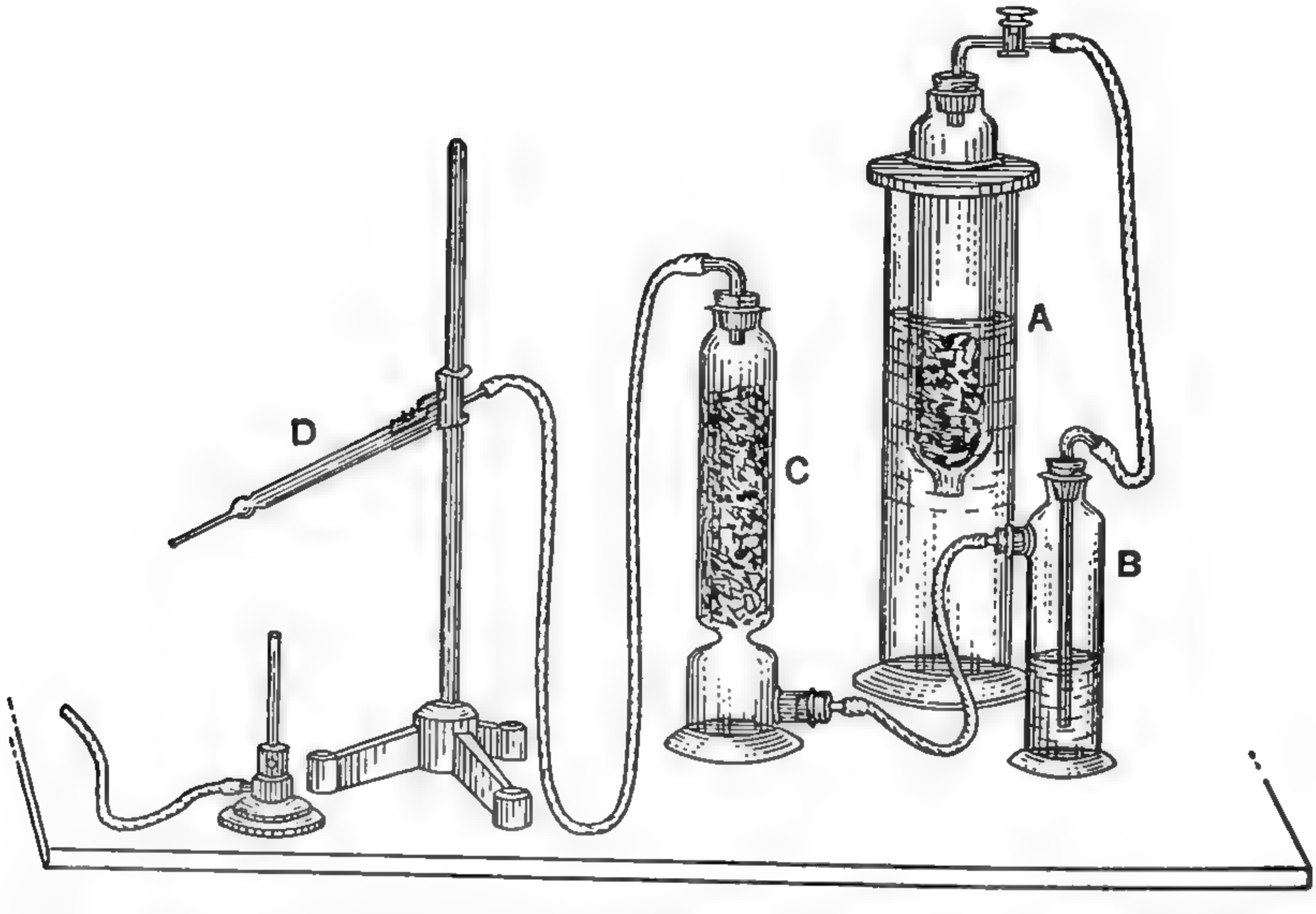


FIG. 283. Apparatus for reducing cuprous oxide to copper. *A*, hydrogen generator; *B* and *C*, gas driers; *D*, filter tube containing cuprous oxide.

heating; otherwise there is danger of explosion. The heating should be continued until all water is expelled from the tube. A desiccator of the form shown in Fig. 284 is convenient for holding filter tubes before weighing.

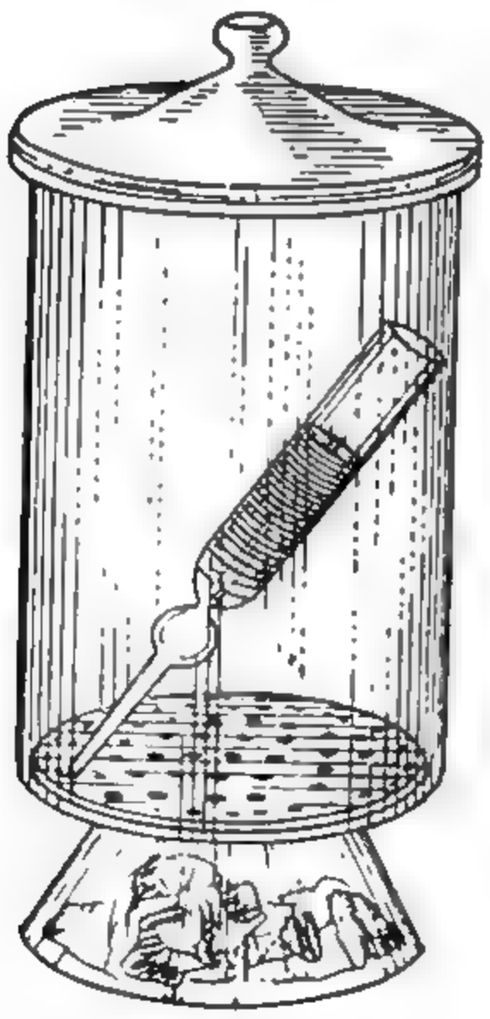


FIG. 284. Desiccator for filter tubes.

Weighing as Copper after Reduction with Alcohol. This method, which was first used by Bruhns, and adapted for use with the Gooch crucible by Staněk,[42] is carried out as follows, according to Wedderburn.[43] Bend the wire ends of a small pipe stem or silica-covered triangle so as to form a tripod support for the crucible, and set the tripod on the bottom of a 400-ml. beaker, preferably of metal. Put into the beaker sufficient strong alcohol (denatured alcohol will answer) to cover the bottom to the depth of about 1 cm. and cover the beaker with a

[42] *Z. Zuckerind. Böhmen*, **32**, 497 (1907/08).

[43] *J. Ind. Eng. Chem.*, **7**, 610 (1915); Meade, "Spencer's Handbook," 7th ed., p. 243, 1929.

watch glass. Heat the alcohol to boiling on a hot plate and continue heating until the vapors begin to condense on the under side of the cover glass. In the meantime the crucible in which the cuprous oxide has been collected is heated to a red heat to destroy all organic matter. When the crucible has cooled to a faint red, place it on the support above the alcohol and replace the cover glass. If the crucible is too hot the alcohol may take fire but the flame may be readily extinguished by blowing directly on top of the cover glass, and no harm is done. Allow the alcohol to continue to boil a moment after putting in the crucible, then remove the beaker from the source of heat. The heat radiated from the crucible will prevent any further condensation of alcohol on the cover glass. The crucible should remain in the covered beaker until cooled a little above the temperature of the alcohol vapor, to prevent oxidation of the copper. About 3 or 4 minutes is sufficient. It may then be taken from the beaker and the cooling finished in a desiccator, preliminary to weighing. If the crucible is quite cold when taken from the beaker it should be moistened with a little pure alcohol and this be burned, holding the crucible in an upright position. After the alcohol burns off the crucible is ready for cooling in the desiccator.

The reduction to metallic copper is almost instantaneous and is complete. The results are usually identical with those by reduction in hydrogen, but under certain conditions there may be a contamination of the copper with carbon from the decomposed alcohol. Such contamination is indicated by a black discoloration of the asbestos pad after solution of the copper in nitric acid, and a correction should be made therefor if the cleaned crucible should show an increase in weight. The alcohol method has been adopted as official by the United States Treasury Department in molasses analyses for customs purposes.

Contamination of Cuprous Oxide Precipitate. The four gravimetric methods thus far described all give accurate results with sugar solutions of high purity, but with impure products the cuprous oxide is likely to be contaminated with mineral and organic impurities, which may affect considerably the accuracy of the determination. When the cuprous oxide is converted into cupric oxide or into copper the organic matter is destroyed by the high temperature employed, but the mineral impurities remain.

The extent of the error in estimating copper from the weight of cuprous or cupric oxide is shown by the following comparative analyses made by Sherwood and Wiley[44] upon a variety of sugar-containing products.

[44] *Bull.* 105, U. S. Bur. Chem., p. 120.

TABLE CVIII

COMPARISON OF METHODS FOR DETERMINING REDUCED COPPER

Material	Reduced Copper		
	From Weight of Cuprous Oxide	From Weight of Cupric Oxide	Volumetric Iodide Method (Low)
	gram	gram	gram
Molasses residuum..	0.3753	0.3594	0.3494
Molasses residuum..	0.3905	0.3634	0.3470
Molasses residuum..	0.2517	0.2348	0.2242
Molasses residuum..	0.3287	0.3130	0.3034
Molasses residuum..	0.3291	0.3134	0.3029
Molasses residuum..	0.2768	0.2698	0.2688
Molasses residuum..	0.2709	0.2620	0.2612
Pure dextrose.....	0.4619	.....	0.4617
Pure dextrose.....	0.2449	.....	0.2444
Pure dextrose.....	0.1251	.....	0.1257
Beer.....	0.0755	.....	0.0753
Beer.....	0.0746	.....	0.0748
Molasses.....	0.4628	.....	0.4520
Corn juice.....	0.3360	.....	0.3134
Malt extract	0.3322	.....	0.3048
Malt extract	0.3160	.....	0.2933
Malt extract	0.2093	.....	0.1934

The results upon the molasses residuum indicate a contamination of the cuprous oxide with organic matter as shown by the differences in copper as calculated from the suboxide and oxide, and with mineral matter as shown by the differences in copper as calculated from the oxide and by the volumetric method.

With solutions of pure sugar and such liquids as beer, where the organic matter consisted largely of carbohydrates, the calculation of copper from the weight of cuprous oxide gave accurate results. In the case of the malt extracts, which contained added peptones, the precipitated cuprous oxide seemed to carry down a considerable amount of albuminoid matter from solution; in the case of the molasses the precipitated copper seemed to be in partial combination with certain nitrogenous bases such as xanthine.

Similar comparisons upon methods of determining copper in the analysis of cane-sugar products are given in Table CXX.[45]

The cuprous oxide may be contaminated even in the absence of non-sugar impurities. Browne has shown that large quantities of sucrose cause the precipitate to come down in impure form and that the contamination increases with the quantity of sucrose as well as of reduced copper. In a mixture of 150 mg. of glucose and 15 g. of sucrose, for

[45] See also Meade and Harris, *Ind. Eng. Chem.*, **8, 504 (1916)**.

instance, the plus error amounts to 10 mg. of copper, compared to weighing as cupric oxide or metallic copper. The estimation of reducing sugars from the weight of cuprous oxide should be entirely abandoned because of the numerous limitations of this procedure.

Determination of Reduced Copper by Electrolysis. This is a gravimetric method which is entirely free from the errors just mentioned. The directions of the Association of Official Agricultural Chemists are as follows.[46]

Decant the hot solution through an asbestos mat in a Gooch crucible, and wash the beaker and precipitate thoroughly with hot water. Transfer the asbestos mat from the crucible to the beaker and rinse the crucible with 14 mm. of nitric acid (1 + 1), allowing the rinsings to flow into the beaker. After the cuprous oxide is dissolved, dilute to 100 ml., heat to boiling, and continue boiling for about 5 minutes to remove oxides of nitrogen. Cool, filter, transfer to a 250-ml. beaker, and dilute to 200 ml. Add 1 drop of 0.1 *N* hydrochloric acid and mix thoroughly.

For the electrolysis use cylindrical electrodes of platinum gauze, 1.5 and 2 inches, respectively, in diameter, and 1.75 inch in height, thoroughly cleaned, ignited, cooled in a desiccator, and weighed. Insert the electrodes in the copper solution so that the surface of the cathode clears the anode by at least 5 mm., and that both electrodes almost touch the bottom of the beaker. Electrolyze with a current of 0.2 to 0.4 ampere until the deposition is complete, usually over night. Without interrupting the current, slowly lower the beaker and at the same time wash the electrodes with a stream of water. Immediately immerse the electrodes in another beaker of water, lower the beaker, and break the current. Rinse the cathode with ethyl alcohol and dry for a few minutes in an oven at 110° C. Cool in the desiccator and weigh.

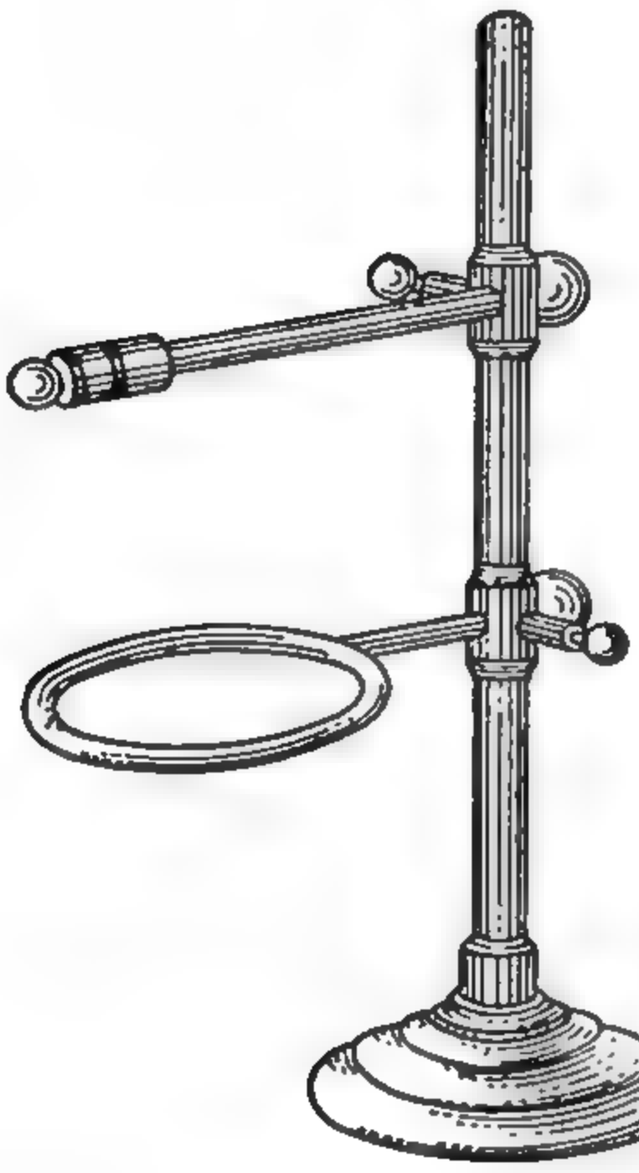
If extreme care is exercised to avoid spattering, the cuprous oxide can be dissolved in the Gooch crucible by allowing the nitric acid to flow down the wall of the crucible. Keep the crucible covered as much as possible with a small watch glass. Collect the filtrate in the beaker and wash the watch glass and the tip of the pipette with a jet of water. Continue as directed above, beginning " dilute to 100 ml. . . ."

To clean the cathode, the deposit is dissolved with nitric acid, followed by washing with water and drying.

The metallic copper may also be deposited on the inside surface of a platinum dish to which the solution of the cuprous oxide in nitric acid is transferred, and which serves as the cathode. A simple apparatus for this procedure is shown in Fig. 285. It consists of an iron base with a glass upright to insulate the anode from the cathode. The ring has three platinum pins to make contact with the platinum dish, and the anode wire is fastened in the clamp provided for this purpose. Con-

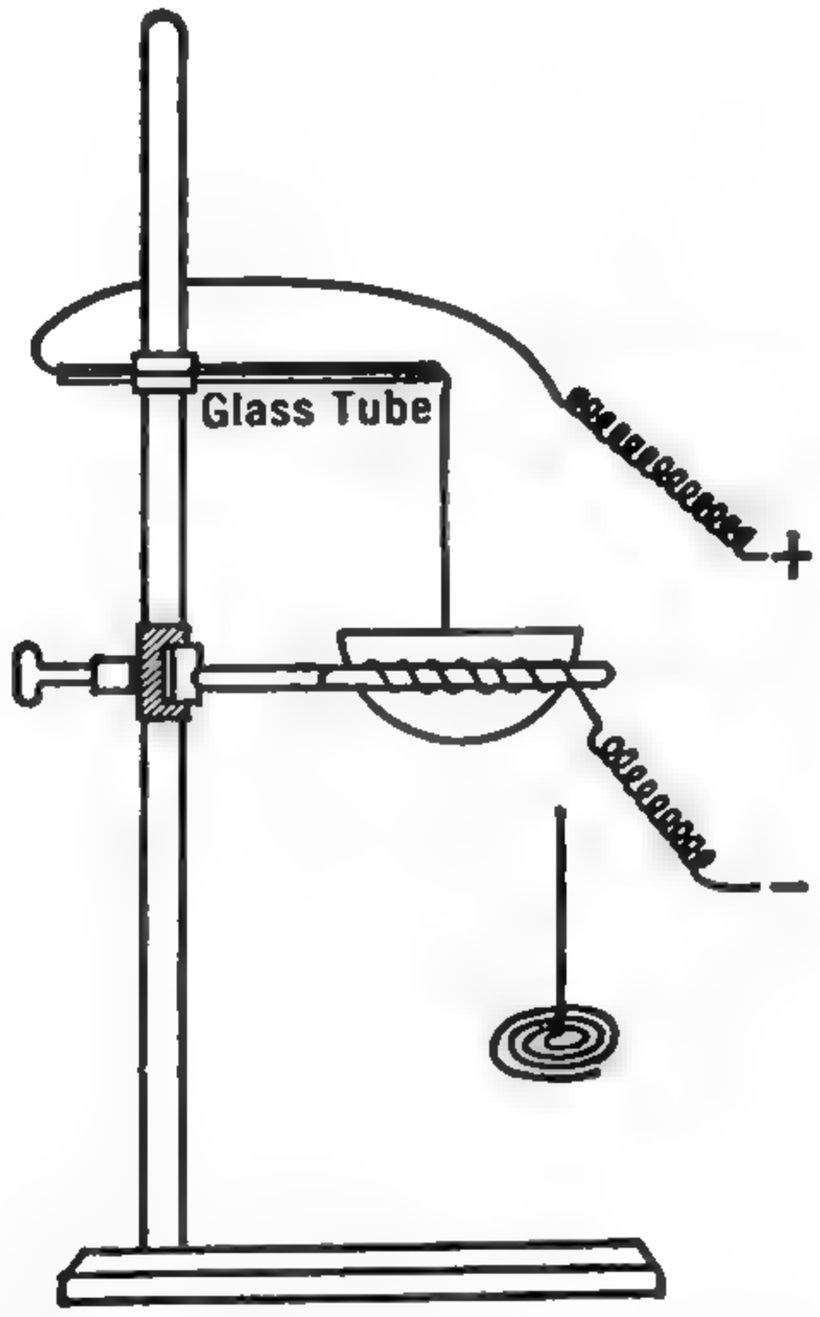
[46] " Methods of Analysis, A. O. A. C.," 5th ed., pp. 502–503, 1940.

nection with the source of current is made through two binding posts. A similar outfit is easily constructed from equipment available in any laboratory, as shown by Meade. (See Fig. 286.) An ordinary iron support is used. The anode is insulated by carrying the wire through a glass tube held in a clamp, and the cathode by a piece of rubber tubing slipped over the iron upright. Contact with the platinum dish is made by winding a spiral of platinum wire around part of the iron ring.



(*Fig. 285, Courtesy of Eimer and Amend.*)

FIG. 285.



(*Fig. 286, Reproduced with permission from Spencer-Meade, "Handbook for Cane-Sugar Manufacturers," p. 245.*)

FIG. 286.

Apparatus for electrolytic determination of copper.

If direct current is available it must be stepped down by means of an appropriate rheostat or by a bank of electric lamps. Otherwise a storage battery is more convenient. If several determinations are to be made at the same time various forms of commercial apparatus are available, some of which are equipped to operate on alternating current as the primary source.

To measure the voltage, the voltmeter is placed parallel with the circuit, the connections being made as close as possible to the anode and cathode, and no other instrument must be interposed. The ammeter is placed, in series, between the voltmeter and the source of current.

Electrolytic Method of Beans and Stillman.[47] In this method the washed cuprous oxide is dissolved with 10 to 20 ml. of dilute nitric

[47] See Quisumbing and Thomas, *J. Am. Chem. Soc.*, 43, 1523 (1921).

acid; the solution is diluted in a beaker to about 200 ml. with distilled water, and 10 ml. of 3 per cent hydrogen peroxide is added. A 50-mesh platinum gauze cathode and a platinum wire spiral anode are then inserted and the solution is electrolyzed at room temperature with a current of 1 ampere and a voltage between 2.5 and 3.5. A mechanical stirrer promotes rapid agitation of the liquid and hastens electrolysis. During the passage of the current 3 per cent hydrogen peroxide is slowly dropped into the solution; when the liquid has become colorless, the sides of the beaker and the cover glass are washed with distilled water, thus raising the level of the solution upon the cathode. The electrolysis is continued until this newly exposed surface shows no deposit of copper. The cathode is then quickly lifted and, without interrupting the current, placed in a beaker of distilled water. It is then washed with 95 per cent alcohol, dried at 100° C. for a few minutes, and after cooling weighed.

Electrolytic Method of Peters. Peters[48] has devised a rapid electrolytic method for the determination of copper, whereby the metal is deposited from an alkaline tartrate solution, such as is used in preparing Fehling's solution. The electrolysis is carried out either in platinum dishes placed upon plates of sheet brass to which the cathode connection is made, or in glass beakers or large test tubes, in which case large cylindrical strips of sheet copper may be used for the cathode. The anode consists of a flat or cylindrical spiral of platinum wire, which should be placed at a distance of 1 cm. or less from the cathode surface. A volume of 10 ml. copper solution (which may be slightly acid or alkaline) is usually taken, to which is added an approximately equal volume of a solution containing 35 g. pure Rochelle salts and 25 g. potassium hydroxide (purified by alcohol) in 100 ml. For copper solutions containing free sulfuric or nitric acid, 2 volumes of the alkaline tartrate solution may be used. From 0.4 to 1.0 ml. of a saturated aqueous potassium cyanide solution is then added according to the amount of copper present; the amount of cyanide solution should be less than sufficient to discharge the blue color. If the copper deposit should be found to be too soft or dark colored, more cyanide should be used; an excess of cyanide, however, greatly lengthens the time for complete deposition of the copper.

In making the determination the direct 110-volt current of a lighting system is used with three incandescent lamps interposed as resistance; the voltage measures 2.6 and the amperage 2.85. During the electrolysis the solution is warmed by a small burner placed under the brass plate to one side of the cathode vessel; if test tubes are used they

[48] *J. Am. Chem. Soc.*, 34, 426 (1912).

are placed upon wire gauze over a small flame. The evolution of gas and the circulation of warm liquid cause a very rapid deposition of copper, which is usually complete in less than 30 minutes. The solution should be covered during electrolysis to prevent loss by spraying.

To determine the completion of electrolysis, Peters recommends the Endemann-Prochazka[49] hydrobromic acid test. One volume of concentrated sulfuric acid is diluted with 2 to 3 volumes of distilled water. About 1 ml. of the dilute acid is placed in a narrow test tube, a few crystals of potassium bromide added, and the whole heated to boiling. A drop of the solution to be tested is then added; as small an amount as 0.007 mg. copper will cause a red color to develop.

If the deposition of copper is complete, the solution in the cathode vessel, without breaking the current, is displaced by a small stream of water until the resistance lamps are extinguished; under this procedure no copper is lost by solution. The electrode containing the deposit of copper is then washed in alcohol and ether, dried, and weighed.

On account of the similarity in composition of the electrolyte employed by Peters to that of the alkaline tartrate solution used in Allihn's method, the process is recommended for the determination of copper in the original Fehling's solution or in the filtrate from the reduced cuprous oxide obtained in the analysis of sugar solutions.

The electrolytic process for determining reduced copper is the most exact of all methods. The determination, however, involves a considerable expenditure of time and for this reason, in sugar laboratories where there is a large amount of routine, it is but little used except for purposes of standardization.

VOLUMETRIC METHODS FOR DETERMINING THE COPPER IN THE FILTERED PRECIPITATE OR IN THE FILTRATE

Several volumetric processes have been devised for determining copper in the filtered precipitate of cuprous oxide. Of these the permanganate, iodide, thiocyanate, and dichromate methods will be described.

Permanganate Method. This method, which is often, but erroneously, referred to as the "Bertrand method," was first proposed by Schwarz,[50] who dissolved the cuprous oxide in ferric chloride solution acidified with hydrochloric acid, and titrated the ferrous iron formed

[49] *Chem. News*, **42**, 8 (1880).

[50] *Ann.*, **84**, 84 (1852).

with permanganate. Mohr[51] substituted ferric sulfate and sulfuric acid for the reagents used by Schwarz, and Müller,[52] in 1898, advocated Mohr's method as a short procedure in reducing sugar determinations.

The reaction between the ferric sulfate and cuprous oxide is expressed as follows:

$$Fe_2(SO_4)_3 + Cu_2O + H_2SO_4 \longrightarrow 2\,FeSO_4 + 2\,CuSO_4 + H_2O$$

Since 1 atom, or 16 parts, of oxygen is required to oxidize the iron reduced by 2 atoms, or 127.14 parts, of copper, and 1 ml. of 0.1 *N* permanganate furnishes 0.0008 g. of active oxygen, then 1 ml. of 0.1 *N* permanganate is equivalent to 0.006357 g. copper. For a solution containing 4.98 g. of potassium permanganate to the liter, 1 ml. will be equivalent to 0.010 g. of copper.

The Mohr-Müller titration method is used extensively in Europe and America, especially in biochemical work. The cuprous oxide is dissolved in a saturated solution of ferric sulfate or ferric alum in 20 per cent sulfuric acid, and the ferrous sulfate formed is titrated with standard permanganate to a permanent pink.

Many investigators have obtained unsatisfactory results with this procedure, especially when using permanganate solutions that are standardized by the usual methods with metallic iron, oxalic acid, or oxalates. It was found necessary to use pure sugars as a standard, to determine the copper in the cuprous oxide by permanganate titration and simultaneously by electrolysis, and to apply a correction factor.

Schoorl and Regenbogen[53] discovered that the cuprous oxide dissolved in the acidified ferric sulfate solution undergoes rapid oxidation, and that this is the principal reason for the discrepancies noted. They also found that correct results are obtained if the cuprous oxide is dissolved in an aqueous solution of ferric sulfate, and that the sulfuric acid must not be added until immediately before the titration with permanganate. In a further study of the method Pick[54] observed that the ferric alum of commerce usually contains ferrous salt; this must first be oxidized to the ferric stage by adding the calculated amount of permanganate solution in preparing the reagent. Pick also suggested acidifying the solution to be titrated with a reagent prepared by dissolving 640 g. crystallized monosodium phosphate in 170 ml. of concentrated sulfuric acid; this produces a liquid of much lighter color, and the end point of the titration with permanganate is much sharper.

[51] *Z. anal. Chem.*, 12, 296 (1873).
[52] *Deut. Zuckerind.*, 23, 790 (1898).
[53] *Z. Ver. deut. Zucker-Ind.*, 67, 563 (1917).
[54] *Z. Zuckerind. čechoslovak. Rep.*, 49, 235 (1924/25).

Sullivan[55] called attention to the fact that the coarser particles of the cuprous oxide dissolve very slowly and that it is necessary to stir very vigorously, preferably with a mechanical stirrer. The solution can also be hastened by breaking up the coarser particles with a flattened glass rod. He also found that the end point of the titration with permanganate can be observed much more sharply if 1 drop of ferrous phenanthroline indicator is added (see p. 779).

The various suggestions for increasing the accuracy of the permanganate titration method have been embodied in the procedure adopted by the Association of Official Agricultural Chemists in 1939.[56] The directions read as follows:

REAGENTS

(*a*) Potassium Permanganate Solution. This is approximately 0.1573 *N*, and contains 4.98 g. per liter. After several days' aging, filter through asbestos or sintered glass. Standardize by one of the following methods:

(1) Transfer[57] 0.35 g. potassium oxalate (dried at 103° C.) to a 600-ml. beaker. Add 250 ml. of sulfuric acid (5 + 95) previously boiled for 10 minutes and cooled to 27° C. ± 3°. Stir until the oxalate is dissolved. Add 29 to 30 ml. of the permanganate solution at the rate of 25 to 35 ml. per minute while stirring slowly. Allow the mixture to stand until the pink color disappears (about 45 seconds). Heat to 55–60° C., and complete the titration by adding permanganate until a faint pink persists for 30 seconds. At last add 0.5 to 1 ml. dropwise, allowing each drop to become decolorized before adding the next.

Determine the excess of solution (usually 0.03 to 0.05 ml.) required to impart a pink color to the same volume of acid, boiled and cooled to 55–60° C. (In potentiometric titrations the correction is negligible if the end point is approached slowly.)

(2) Transfer about 0.3 g. of arsenic trioxide (dried at 110° C.) to a 400-ml. beaker. Add 10 ml. of a cool solution of sodium hydroxide (20 per cent) and allow to stand until dissolved, stirring occasionally. Add 100 ml. of water, 10 ml. of hydrochloric acid (sp. gr. 1.18), and 1 drop of 0.0025 *M* potassium iodide solution. Titrate with the permanganate solution until a faint pink color persists for 30 seconds, adding the last 1 to 1.5 ml. dropwise and allowing each drop to become decolorized before adding the next. Determine, by a blank test with all the reagents except the arsenic trioxide, the volume of permanganate (usually about 0.03 ml.) required to duplicate the pink color of the end point. (The end point can also be taken with ferrous phenanthroline indicator, in which case 1 drop of a 0.025 *M* solution of the indicator is added as the end point is approached.) Determine the blank correction. The titration can also be conducted potentiometrically.

[55] *J. Assoc. Official Agr. Chem.*, **18**, 382 (1935); **19**, 125 (1936).
[56] "Methods of Analysis, A. O. A. C.," 5th ed., pp. 501–502, 1940.
[57] Fowler and Bright, *Bur. Standards J. Research*, **15**, 493 (1935).

(*b*) Ferric Sulfate. Dissolve 135 g. of ferric ammonium sulfate or 55 g. of anhydrous ferric sulfate in water and fill to 1 liter. Determine the quantity of ferric sulfate in the stock supply by strong ignition to ferric oxide. Acidify 25 ml. of the reagent with 10 ml. of 4 *N* sulfuric acid and titrate with the permanganate. Add to the remaining (unacidified) stock solution the calculated volume of permanganate.

(*c*) Ferrous Phenanthroline Indicator. Dissolve 0.7425 g. of orthophenanthroline monohydrate in 25 ml. of 0.025 *M* ferrous sulfate solution (6.95 g. of $FeSO_4 \cdot 7H_2O$ in 1 liter).

DETERMINATION

Filter the cuprous oxide through a Gooch crucible and wash the beaker and precipitate thoroughly. Transfer the asbestos mat to the same beaker with the aid of a glass rod. Add 50 ml. of the ferric sulfate reagent and stir vigorously until the cuprous oxide is completely dissolved. Examine for complete solution, holding the beaker above the level of the eye. Add 20 ml. of 4 *N* sulfuric acid and titrate with the standard permanganate. As the end point is approached, add 1 drop of the ferrous phenanthroline indicator. At the end point the brownish solution changes to green.

Volumetric Iodide and Thiosulfate Method. Of the many modifications of the method for determining copper with iodide and thiosulfate, the Association of Official Agricultural Chemists uses that described by Low,[58] with a slight change proposed by Popoff and introduced by Jackson.[59] It is carried out as follows:[60]

Standard Thiosulfate Solution. Prepare a solution containing 39 g. of pure $Na_2S_2O_3 \cdot 5H_2O$ in 1 liter. Weigh accurately 0.2–0.4 g. of pure copper and transfer to a 250-ml. Erlenmeyer flask roughly graduated by marks at 20-ml. intervals. Dissolve the copper in 5 ml. of a mixture of equal volumes of nitric acid and water, dilute to 20 or 30 ml., boil to expel the red fumes, add a slight excess of strong bromine water, and boil until the bromine is completely driven off. Cool, and add sodium hydroxide solution with agitation until a faint turbidity of cupric hydroxide appears (about 7 ml. of a 25 per cent sodium hydroxide solution is required). Discharge the turbidity with a few drops of acetic acid and add 2 drops in excess. Prepare a solution of 42 g. of potassium iodide of 100 ml. of solution made very slightly alkaline to avoid formation of hydriodic acid and its oxidation.

It is essential for the thiosulfate titration that the concentration of potassium iodide in the solution be carefully regulated. If the solution contains less than 320 mg. of copper, at the completion of the titration 4.2–5 g. of potassium iodide should have been added for each 100 ml. of total solution. If greater

[58] *J. Am. Chem. Soc.*, **24**, 1082 (1902).
[59] *J. Assoc. Official Agr. Chem.*, **12**, 38, 166 (1929).
[60] " Methods of Analysis, A. O. A. C.," 5th ed., p. 501, 1940.

quantities of copper are present, add the potassium iodide solution slowly from a burette with constant agitation in amounts proportionately greater.

Observe the volume of the copper solution and add 1 ml. of potassium iodide solution for each 10 ml. of the solution undergoing titration. Titrate at once with the thiosulfate solution until the brown color becomes faint. Again observe the volume and add an additional volume of potassium iodide to make the required concentration, noting from the volume of the thiosulfate the approximate copper content of the solution. Add sufficient starch indicator to produce a marked blue coloration. Continue the titration cautiously until the color changes toward the end to a faint lilac. As the end point is approached, add the thiosulfate in fractions of drops, allowing the precipitate to settle slightly after each addition.

DETERMINATION

Wash the precipitated cuprous oxide, cover the Gooch crucible with a watch glass, and dissolve the oxide by means of 5 ml. of nitric acid (1 + 1) directed under the watch glass with a pipette. Collect the filtrate in a 250-ml. Erlenmeyer flask which is roughly graduated by marks at 20-ml. intervals, and wash the watch glass and Gooch crucible free from copper. Proceed as directed under " Standard Thiosulfate Solution," beginning with " boil to expel the red fumes . . ."

Since 1 atom, or 63.57 parts, of copper liberates 1 atom, or 126.92 parts, of iodine and 1 ml. of $N/10$ thiosulfate solution (24.8 g. $Na_2S_2O_3 + 5\,H_2O$ to 1000 ml.) reacts with 0.01269 g. I, then 1 ml. $N/10$ thiosulfate corresponds to 0.00636 g. copper. For a solution containing 39 g. of pure sodium thiosulfate to the liter, 1 ml. will be equivalent very closely to 0.010 g. of copper. The above reaction is reversible and the results of the determination vary somewhat according to concentration of acid, excess of reagents, temperature, and other conditions. It is, therefore, important always to standardize the thiosulfate solution against pure copper under the exact conditions which are followed in analysis.

According to Foote and Vance,[61] practically theoretical results for copper may be obtained by dissolving 2 g. of ammonium thiocyanate in the reaction mixture toward the end of the titration after the starch has been added. This converts the cuprous iodide, which is usually colored by adsorbed iodine, into perfectly white and less soluble cuprous thiocyanate.

Kendall's Modification of the Iodide Method. The removal of the nitrous acid, formed in dissolving the copper, is the chief difficulty in the iodide method. Kendall[62] has modified the method by removing

[61] *J. Am. Chem. Soc.*, **57, 845 (1935).**
[62] *J. Am. Chem. Soc.*, **33, 1947 (1911).**

the nitrous acid with hypochlorite, the free chlorine which is evolved being afterwards removed with phenol.

The cuprous oxide, after filtering and washing upon a Gooch crucible, is dissolved in 10 to 15 ml. of 30 per cent nitric acid into a 300 ml. Erlenmeyer flask. The volume of solution and washings should be between 50 and 60 ml. with an acidity of 4 to 5 ml. concentrated nitric acid; 5 ml. of sodium hypochlorite solution is then added of such strength that the iodine liberated by 5 ml. is equivalent to 30 ml. of $N/10$ thiosulfate. The solution is allowed to stand 2 minutes, when 10 ml. of a 5 per cent colorless phenol solution is quickly added. The chlorine gas above the liquid is removed by blowing into the flask, and the sides are washed down with a jet of water. The solution is then made slightly alkaline with sodium hydroxide and acidified with acetic acid; 10 ml. of 30 per cent potassium iodide solution is then added and the free iodine titrated with standard sodium thiosulfate, as under Low's modification, using soluble starch as indicator. The thiosulfate is previously standardized against pure copper under the same conditions as those of the method.

In working with known weights of copper between 20 and 340 mg., Kendall found the error of his method to exceed in no case 0.3 mg., but Quisumbing and Thomas[63] state that Kendall's method gives a poor end point.

Peters's Modifications of the Iodide Method. Peters[64] has found that boiling the nitric acid solution of copper in the presence of talcum powder will remove completely all lower oxides of nitrogen and leave the solution, after cooling and diluting, in suitable condition for titration. The copper, or its compound, is dissolved in an Erlenmeyer flask in the least possible volume of concentrated nitric acid, to which one-half its volume of water has been added; 5 to 10 ml. of dilute acid is sufficient for 0.5 g., or less, of copper. After solution 15 to 25 ml. of distilled water and a little pure powdered talcum are added, and the mixture boiled vigorously for 5 to 10 minutes. After cooling to room temperature distilled water is added and 10 ml. of a saturated potassium iodide solution, the dilution being so regulated that the final volume of liquid at the end of the thiosulfate titration is about 120 ml.

Peters has also employed the iodide method in the determination of copper in the alkaline tartrate solutions, or filtrates, occurring in sugar analysis. In the modification employed, 20 ml. of Allihn's alkaline tartrate solution, 20 ml. of Fehling's copper sulfate solution, and 20 ml.

[63] *J. Am. Chem. Soc.*, 43, 1503 (1921).
[64] *J. Am. Chem. Soc.*, 34, 422 (1912).

of water (as in a blank determination), or of the aqueous reducing-sugar solution, were taken, making the total volume for reduction always 60 ml. After the reduction the cuprous oxide is filtered and washed, and the filtrate, which has a volume of 70 to 75 ml., is acidified with 4 to 5 ml. of concentrated sulfuric acid. After cooling to about 20° C., 10 ml. of saturated potassium iodide is added and the solution is titrated with standard thiosulfate in the usual way.

The end point of the titration in the iodide method is best determined according to Peters by noting the point at which a drop of the thiosulfate solution ceases to produce a perceptible white area upon the quiet surface of the titration liquid. The thiosulfate solution must be standardized against pure copper under the exact conditions of the analysis.

Potassium iodide is an expensive reagent, and, where many determinations of copper are made by this method, the waste titration liquids and cuprous iodide precipitates should be saved for recovery of the iodine.

Instead of talcum powder, 0.5 g. of urea[65] may be added to the solution of the cuprous oxide in strong nitric acid, to eliminate the nitrous acid fumes. The solution is then heated to boiling, and cooled. Ammonia of 6 *N* strength is added until a white precipitate forms. This is redissolved with 6 *N* acetic acid, and 5 ml. is added in excess. The determination is completed as described under Jackson's modification of Low's method (p. 779).

Volumetric Thiocyanate Method (Volhard-Pflüger).[66] The following solutions are required: (*a*) 0.1 *N* silver nitrate solution, (*b*) 0.1 *N* ammonium thiocyanate solution, (*c*) a cold saturated solution of sulfur dioxide (SO_2) in water, (*d*) nitric acid of sp. gr. 1.2, free from nitrous acid, (*e*) a saturated solution of ferric alum, (*f*) normal sulfuric acid solution.

The filter tube, or Gooch crucible, containing the cuprous oxide is weighed and the approximate amount of copper determined. The cuprous oxide is then dissolved from the asbestos with nitric acid; the solution is treated with a slight excess of normal sulfuric acid solution (*f*) necessary to convert all the copper into copper sulfate and evaporated to dryness. The copper sulfate is then dissolved in water and washed into a 300-ml. graduated flask. Sodium carbonate solution is added to the point of turbidity, and then 50 ml. of the sulfurous acid reagent (*c*). The solution is boiled for 1 minute, and then 0.1 *N* thio-

[65] Koelsch, *Chem.-Ztg.*, **37**, 753 (1913); Pozzi-Escot, *Ann. chim. anal.*, **18**, 219 (1913); Hill, *Ind. Eng. Chem., Anal. Ed.*, **8**, 200 (1936).

[66] *Pflügers Archiv*, **69**, 423 (1898).

cyanate (*b*) is added until there is an excess of about 5 ml. above the calculated amount necessary for precipitating the copper as cuprous thiocyanate [$Cu_2(SCN)_2$]. The solution is then cooled, made up to 300 ml., shaken, and filtered through dry filter paper. Should the first runnings appear turbid, they are returned to the filter; 100 ml. of the clear filtrate is diluted with 100 ml. of water, 50 ml. of nitric acid (*d*) and 10 ml. of ferric-alum solution (*e*) are added, and the solution titrated with 0.1 *N* silver nitrate (*a*) until the red color is discharged. The addition of silver solution is continued to the next even number of milliliters and then the solution titrated back with 0.1 *N* thiocyanate until the white liquid just begins to turn pink.

Let *A* be the milliliters of 0.1 *N* thiocyanate added to the 300 ml. of solution, *B* the milliliters of 0.1 *N* silver nitrate added to the 100 ml. of filtrate, and *C* the milliliters of 0.1 *N* thiocyanate to titrate back excess of *B*.

Since 1 ml. 0.1 *N* thiocyanate = 6.357 mg. copper then the total milligrams of copper (Cu) are found by the formula $Cu = 6.357 (A + 3C - 3B)$. The thiocyanate solution should be standardized against pure copper under the conditions of analysis, as in the iodide methods.

Volumetric Dichromate Method. In this procedure, introduced by Jackson and Mathews[67] in connection with their method for determining fructose (p. 824), the cuprous oxide is dissolved in an excess of dichromate solution, and the excess titrated back with ferrous iron; 7.7135 g. of recrystallized potassium dichromate is dissolved to 1 liter; 1 ml. of this solution is equivalent to 10 mg. of copper. A solution of about the same normality is prepared by dissolving 61.8 g. of ferrous ammonium sulfate hexahydrate and 5 ml. of concentrated sulfuric acid to 1 liter. This solution is not quite stable, losing about 0.3 per cent of its reducing power per day. It must therefore be standardized against the dichromate solution during each series of determinations. The end point is determined by means of 1 drop of 0.025 *M* orthophenanthroline ferrous sulfate, which changes from blue to intense red with the slightest excess of ferrous ion.[68] The electrometric method of Forbes and Bartlett[69] may also be used for determining the end point.

The procedure is described by Jackson and McDonald as follows:[70] Estimate the volumes of dichromate, ferrous sulfate, hydrochloric acid, and water that will give an assured excess of dichromate and yield a

[67] *Bur. Standards J. Research*, **8**, 403 (1932).
[68] *J. Am. Chem. Soc.*, **53**, 3908 (1931).
[69] *J. Am. Chem. Soc.*, **35**, 1527 (1913).
[70] *J. Assoc. Official Agr. Chem.*, **18**, 172 (1935).

concentration of about 2 *N* acid in about 200 ml. of final volume. An error of 5 per cent, or in most cases even 10 per cent, can be tolerated. Fill graduated cylinders with the required volumes of water and acid.

Collect the precipitated cuprous oxide on a Gooch crucible and wash thoroughly. Detach the mat with the glass rod and transfer to the reaction beaker. Add a small volume of water and disintegrate the mat. Pipette accurately a volume of standard dichromate in excess of the quantity required to oxidize the cuprous oxide. In general the expected weight of copper will be known or can be roughly estimated, but in any case a sufficient volume must be added to supply an assured excess. Add rapidly the whole required volume of hydrochloric acid with continuous stirring, and continue to stir until all cuprous oxide is dissolved. Immerse the crucible in the solution and be assured that the adhering cuprous oxide is dissolved. Remove the crucible with the glass rod and wash it with the water from the graduate. Add 1 drop of phenanthroline indicator and titrate with the ferrous sulfate to the permanent appearance of the brown ferrous phenanthroline complex. As the end point is approached the brown color appears and fades as each of the last few drops is added, and the ferrous sulfate must be added until the color is permanent, the additions finally being in fractions of drops.

Determine the ratio of concentrations of ferrous sulfate and dichromate and thence compute the volume of dichromate required for the oxidation of cuprous oxide. This volume multiplied by 10 gives directly the number of milligrams of copper reduced.

Stegeman and Englis[71] have recommended ceric sulfate for dissolving the cuprous oxide. The excess is titrated back with ferrous sulfate and phenanthroline indicator, as in Jackson's method. Ceric sulfate is an expensive reagent, and its use in this method requires further study.

Jackson and McDonald[72] consider the thiosulfate method to be the most precise of the volumetric methods. The dichromate method is only slightly less precise, but more rapid and convenient. Opinion is still divided about the permanganate method, but the modification adopted by the Association of Official Agricultural Chemists appears to be quite reliable. In the analysis of impure products the iodide thiosulfate method is to be preferred because organic impurities occluded in the cuprous oxide are likely to affect the titration in the permanganate method, and to a lesser extent in the dichromate method.[73]

[71] *Trans. Ill. State Acad. Sci.*, **27, 75** (1934).

[72] *Loc. cit.*

[73] Stegeman and Englis, *J. Assoc. Official Agr. Chem.*, **19,** 480 (1936).

Various methods have been proposed to determine the copper in the precipitated cuprous oxide colorimetrically. Stare[74] dissolves the precipitate in hydrochloric acid, oxidizes the copper to the bivalent form with hydrogen peroxide, adds an excess of ammonia, and compares the solution with known standards in a colorimeter. For more exact results the Pulfrich photometer (p. 597) or a photoelectric colorimeter (p. 600) may be employed, with appropriate light filters.

Dubourg and Goldstein[75] have observed that freshly precipitated cuprous oxide dissolves readily in a mixture of 10 ml. of 20 per cent ammonium hydroxide and 10 ml. of hydrogen peroxide of 12 volume per cent. The solution is diluted to 30 ml. and compared colorimetrically with known standards.

Determination of Unreduced Copper by the Volumetric Cyanide Method. Of other volumetric processes which are used for determining reduced copper may be mentioned the well-known cyanide method. The unreduced copper in the filtrate from the cuprous oxide is titrated with standard potassium cyanide solution until the blue color disappears. The difference between the copper in the volume of Fehling's solution taken, and that found in the filtrate after reduction, is the amount of copper reduced by the sugar.

The cyanide method has been recommended by Hörne[76] for rapid routine work in the sugar factory. Twenty milliliters of Soxhlet solution and 20 ml. of sugar solution containing from 30 to 60 mg. invert sugar are used. The reduction is carried out as usual, the volume of the reaction mixture made up with cold water to 100 ml., and the temperature noted. The liquid is poured on a filter paper, and the equivalent of 50 ml. of filtrate, corrected for temperature, is titrated with 5 per cent potassium cyanide solution which has previously been standardized against the Soxhlet solution. The 50 ml. of hot solution is measured out with a special pipette which automatically applies the necessary temperature correction.

Stolle[77] advocates the addition of a solution containing 200 ml. of ammonia and 100 g. pure ammonium carbonate per liter to the Soxhlet solution before titration with potassium cyanide because this affords a sharper end point.

The copper remaining in the filtered solution after reduction may also be determined by any of the well-known colorimetric methods for the estimation of copper, and deducted from the total present before

[74] *Bull. assoc. chim.*, **53**, 456 (1936).
[75] *Bull. assoc. chim.*, **55**, 543 (1938).
[76] *Louisiana Planter*, **81**, 1 (1928).
[77] *Z. Ver. deut. Zucker-Ind.*, **51**, 111 (1901).

reduction. Various procedures based on this principle have been proposed,[78] but none of them has come into extended use.

Conditions Affecting the Precipitation of Cuprous Oxide by Reducing Sugars

In addition to the causes of error in determining reduced copper, there are a number of factors which affect the accuracy of the analytical methods belonging to this class.

Purity of Reagents. A frequent cause of inaccuracy in determining sugars by the methods of copper reduction is the presence of organic or mineral impurities in the Fehling's solution. The copper sulfate, the caustic alkali, and especially the Rochelle salt should be of the purest quality. The copper sulfate and alkali tartrate solutions should be filtered separately through glass wool or asbestos, and the mixed reagent should be perfectly clear and show no trace of cuprous oxide after boiling.

A blank determination should be made upon each fresh lot of solution; the crucibles or filter tubes used in the blank test should show no increase in weight under the conditions of experiment followed in analysis. Quisumbing and Thomas[79] found that, when 50 ml. of Soxhlet reagent, prepared from specially purified reagents and diluted with water to 100 ml., is heated to boiling, it gives a precipitate containing 4.3 mg. copper in 10 minutes. At 80° C. no precipitate whatever is formed within 30 minutes. Bruhns[80] observed that when the Rochelle salt is of the highest purity the amount of copper reduced during 2 minutes' boiling is negligible.

Adsorption of Fehling's Solution by Filtering Materials.[81] Amick has noted that Fehling's solution is adsorbed by filter paper or asbestos, and cannot be removed by washing with water. This introduces an error in all those methods where such filtration is practiced. Furthermore, in the permanganate titration method (p. 776) the adsorbed tartrate is dissolved by the ferric salt solution, and slightly oxidized by the permanganate. From the standpoint of practical sugar analysis these errors are not important, however, as long as the copper reagent is standardized under the same conditions in which it is used. Nevertheless, Amick's results present an argument in favor of those methods where the copper is determined without filtration.

[78] E. M. Emmert, *J. Assoc. Official Agr. Chem.*, **15, 327** (1932).
[79] *J. Am. Chem. Soc.*, **43, 1503** (1921).
[80] *Centr. Zuckerind.*, **36, 1452** (1928).
[81] *J. Phys. Chem.*, **31, 1441** (1927).

Composition of Copper Reagent. The effect of the concentrations of the three principal constituents of Fehling's solution has been studied in great detail by Quisumbing and Thomas.[82] As the concentration of the alkali is increased the amount of copper reduced in a given time by a given quantity of reducing sugar rises rapidly, reaches a maximum at about 1.6 *N* for sodium hydroxide (65 g. per liter of mixed reagent) and at about 2 *N* for potassium hydroxide (112 g. per liter), and then descends slowly. When alkali carbonates are used instead of the hydroxides, the curves show first a gradual rise, with a maximum at about 6 *N*, and then a gradual descent.

At the optimum alkalinity of 1.6 *N* sodium hydroxide the highest reducing action is obtained when the ratio of copper to alkali in the reagent is as about 1 : 5; below and above this ratio the amount of copper precipitated decreases. The effect of the concentration of the Rochelle salt is slight, but the 173 g. prescribed by Soxhlet gives the best results.

Rate of Reduction. This subject has been studied by a number of investigators, notably Bates and Jackson,[83] Quisumbing and Thomas,[84] Shaffer and Somogyi,[85] and Spengler and Tödt.[86] At any given temperature to which the solution is heated, the amount of copper precipitated from Fehling's solution by reducing sugars rises rapidly at first, more so at high temperatures, and then increases more slowly to a maximum. The rates for different reducing sugars are not the same. The monosaccharides as a group show a more rapid rate than the reducing disaccharides, like lactose and maltose. If reduction of Fehling's solution with invert sugar is carried out at the boiling point, the maximum reduction is nearly reached when the solution begins to boil. If it is desired to obtain nearly maximum reduction with lactose or maltose, the time of heating must be increased, or the composition of the copper reagent must be changed.

Sucrose, which is not classed as a reducing sugar, is nevertheless gradually hydrolyzed, especially by strongly alkaline copper reagents such as Soxhlet's. Its reduction curve is very different from that of the typical reducing sugars, rising very slowly and steadily in an almost straight line as the boiling is continued. The reducing effect of sucrose is further discussed on pp. 803–806.

Experiments by Shaffer and Somogyi, in which copper tartrate solu-

[82] *Loc. cit.*
[83] *Bur. Standards Sci. Paper* 268, 1916.
[84] *J. Am. Chem. Soc.*, 43, 1503 (1921).
[85] *J. Biol. Chem.*, 100, 695 (1933).
[86] *Z. Ver. deut. Zucker-Ind.*, 83, 833 (1933).

tions containing 0.1 *M* sodium hydroxide and 0.6 to 1 *M* carbonate solutions with varying ratios of carbonate to bicarbonate were compared with one another, have shown that, the lower the alkalinity, the slower is the copper reduction, but the higher is the final amount of copper reduced. With short periods of heating higher alkalinity gives higher reduction, but maximum reduction is obtained at the lowest alkalinity if the heating is sufficiently prolonged. The maximum reduction varies inversely with the logarithm of the ratio of carbonate to bicarbonate. However, at an equal ratio between carbonate and bicarbonate, an increase in the total carbonate content from 0.6 to 1 *M* gave a rise in the maximum reduction.

When the total carbonate content is kept constant, the velocity of reduction is a linear function of the log of the carbonate-bicarbonate ratio; an increase in total carbonate content slows up the rate of reduction.

Degree of Dilution. The effect of varying the volume of Fehling's solution with respect to that of sugar solution has also been studied by Spengler and Tödt, who found that 15 mg. of invert sugar alone reduces, under certain experimental conditions, 28.75 mg. of copper from 50 ml. of Soxhlet solution, and 26.7 mg. from 20 ml., or about 7 per cent less. But when 10 g. of sucrose is present in addition to the invert sugar, the amount of copper precipitated is diminished from 57.3 to 40.0 mg., or almost 30 per cent, and with 10 g. sucrose alone from 32.3 to 13.6 mg., that is, 58 per cent.

Temperature of Heating and Atmospheric Pressure. The time of heating being kept constant, a rise in the temperature increases the amount of reduced copper. For this reason a longer heating period is employed when the reduction is carried out at temperatures below the boiling point.

Since the boiling point is affected by the atmospheric pressure, differences in altitude above sea level have been suggested by Traphagen and Cobleigh[87] as a cause of differences in determining reducing sugars. Rosenkranz[88] has studied the influence of pressure upon the reducing power of invert sugar with the results shown on p. 789. The experiments were made in an Erlenmeyer flask with a two-hole stopper. A thermometer was inserted through one hole, and a reflux condenser through the other. The condenser carried at its upper end a T-tube, one branch of which was connected with a mercury manometer, and the other with a water-jet pump for creating either pressure or vacuum.

[87] *J. Am. Chem. Soc.*, **21**, 369 (1899).
[88] *Z. Ver. deut. Zucker-Ind.*, **61**, 426 (1911).

Pressure	Temperature of Boiling	25 ml. Invert-Sugar Solution plus	
		25 ml. Water. 50 ml. Fehling's Solution	25 ml. 10% Sucrose Solution. 50 ml. Fehling's Solution
millimeters	°C.	mg. Cu	mg. Cu
775	103–105	236.5	260.4
600	90– 96	232.5	244.9
760	103–104	235.6	277.7
925	109–110	236.1	296.3

The results show for pure invert sugar a slight tendency towards increase in reducing power with increase in pressure. When sucrose is present the increase in pressure causes a marked increase in the amount of reduced copper, owing to greater hydrolysis of the sucrose.

Quisumbing and Thomas found much greater effects of temperature differences than did Rosenkranz; the reducing effect of 100 and 150 mg. of glucose on Soxhlet solution was 2 per cent higher at 760 mm. pressure than at 753 mm. These results show that reducing-sugar tables constructed for methods in which the solution is either boiled directly or heated in a boiling-water bath at sea level do not give correct results when the work is performed at higher altitudes, and that even at one and the same elevation variations in atmospheric pressure may introduce considerable errors. The methods in which the reduction is carried out at a specified temperature, like those of Quisumbing and Thomas, or of Saillard, are free from these errors.

In this connection should also be mentioned the effect of overheating, due to boiling retardation when new beakers or flasks with smooth surfaces are used, and both copper and sugar solution have been carefully filtered. Under these conditions the liquid may be heated considerably above its normal boiling point without generating steam bubbles. This is an important source of error when small quantities of reducing sugars are determined in the presence of a large excess of sucrose; this subject has been extensively studied by a number of investigators, and is more fully discussed on p. 808.

Precipitation of Cuprous Oxide in Colloidal Form. In the analysis of low-grade molasses and various biological products the cuprous oxide precipitate is sometimes not red in color, but yellow, imparting to the copper solution a greenish appearance. Fischer and Hooker[89] have explained this phenomenon on the basis of colloid-chemical principles. In the presence of protective colloids, such as certain gums, the

[89] *Intern. Sugar J.*, 21, 76 (1919).

cuprous oxide is obtained in a highly dispersed state when its color is yellow. Under these circumstances the precipitate is liable to run through the filter and to deposit in the filtrate upon standing. Schuette[90] has found this to happen particularly with Ost's solution. It also occurs frequently in Kraisy's method for the determination of small quantities of invert sugar in high-grade cane or beet sugars (p. 842), where the sucrose itself acts as a protective colloid. In all these cases it is best not to use a filtration method, but to determine the copper in the cuprous oxide precipitate by titration in the presence of the excess copper reagent.

Surface Area of Solution. The diameter of the vessel in which the Fehling's solution is heated has been found to influence the amount of reduced copper. With wide beakers, which expose a larger area of solution to the air, more cuprous oxide is lost by oxidation (through being redissolved in the alkaline tartrate solution) than in narrower beakers. Kjeldahl has eliminated the error due to oxidation by making the reduction in an atmosphere of hydrogen or of oxygen-free illuminating gas. It can also be overcome almost completely by covering the beaker with a watch glass, as recommended by Munson and Walker; or, in the boiling methods, by using Erlenmeyer flasks in which the constantly escaping steam keeps out the air, as in the method of Lane and Eynon.

On the other hand, Quisumbing and Thomas have found that, the larger the lateral surface of the vessel with which the liquid comes in contact, the greater is the amount of copper reduced, through a catalytic action of the glass.

Under the same set of conditions the errors due to surface oxidation and to the catalytic effect of the glass are constant, and the discrepancies due to these causes are eliminated by making the reduction always in vessels of the same size and shape. A 350- or 400-ml. beaker of resistance glass, like Pyrex or Jena, 7–8 cm. in diameter, is quite generally used.

Effect of Admixtures on the Reducing Power of Sugars. Impurities which may affect the determination of reducing sugars are usually removed as far as possible by appropriate clarifying agents (see p. 882). But other substances may be present, sugars as well as non-sugars, which are not removed by the usual clarifying agents, like the neutral lead acetate generally employed in sugar-factory products. The effect of sucrose on the reducing power of glucose, etc., is discussed in detail on pp. 803–806, but even the different reducing sugars mutually affect their reducing power, as has been shown by Quisumbing and Thomas,

[90] *J. Assoc. Official Agr. Chem.*, **13, 175 (1930)**.

by Lane and Eynon,[91] and by Zerban and Sattler.[92] In exact work these influences must be taken into account.

Herlesová[93] found that nitrogenous substances, such as amino acids and their amides, and purine bases which occur in molasses and are only partially removed by neutral lead acetate, lower the quantity of precipitated cuprous oxide, acting as ammonia does in the Pavy copper reagent. Similar observations have been reported by Lundin.[94] Cyanides which may appear in the analysis of glucosides also cause low results.[95] Aldehydes, such as formaldehyde which may be present as a preservative, or acetaldehyde which may have been formed by fermentation, have the opposite effect, causing too high results. These few examples will suffice to show that the chemist must guard against errors which may arise from these sources and overcome their effects by appropriate measures.

SPECIAL REDUCTION METHODS WITH COPPER REAGENTS CONTAINING CAUSTIC ALKALI

Modifications of Allihn's Method. Allihn's method gives the most accurate results upon sugar solutions containing 0.4 to 1.0 per cent glucose, i.e., 0.10 to 0.25 g. glucose in the 25 ml. of solution. When less than 50 mg. of glucose is present the method is likely to show wide discrepancies in the hands of different chemists. Several modifications of Allihn's method, involving a longer period of heating, have been devised for the purpose of increasing the accuracy of the determination with dilute sugar solutions.

Pflüger's Method. Pflüger,[96] who used the same reagents and volumes of solutions as in Allihn's method, modified the determination by heating the mixed sugar and Fehling's solutions (145 ml. in all) in a boiling-water bath for exactly 30 minutes and then diluting with 130 ml. of cold water before filtering. The cuprous oxide is filtered upon asbestos and, after washing and drying, the weight of precipitate determined. Owing to the frequent occurrence of impurities in the cuprous oxide, especially when working with fluids or extracts of animal origin, Pflüger advised making also a direct determination of the copper by means of the thiocyanate method.

[91] *J. Soc. Chem. Ind.*, **42**, 32T (1923).
[92] *Ind. Eng. Chem., Anal. Ed.*, **2**, 307 (1930).
[93] *Z. Zuckerind. čechoslovak. Rep.*, **54**, 1 (1929/30).
[94] *Biochem. Z.*, **207**, 91 (1929).
[95] Hérissey and Chalmeta, *J. pharm. chim.*, [8], **8**, 393 (1928).
[96] *Pflügers Archiv*, **69**, 399 (1898).

Koch and Ruhsam's[97] Method. In this modification the same reagents and volumes of solutions are used as in Allihn's and Pflüger's methods. The mixed sugar and Fehling's solutions (145 ml. in all) are first brought to a boil and then set in a boiling-water bath for exactly 30 minutes. The solution without diluting is then filtered through asbestos in a Gooch crucible and the reduced copper determined by any of the usual methods.

Koch and Ruhsam's modification was designed especially for determining glucose in tannin extracts, etc.

The modifications of Allihn's method, using 30-minute heating, are considerably more accurate than the original process upon dilute glucose solutions and are useful for determining small amounts of sugar in urine, tannin extracts, and other animal and vegetable substances of low glucose content. When, however, the 25 ml. of sugar solution contains over 0.10 g. of glucose, Allihn's original method of 2-minute boiling may be followed with perfect safety, and with a considerable economy of time. The fact that more copper is reduced upon longer heating does not affect the accuracy of the method, since the tables were standardized under exactly similar conditions.

The methods of Pflüger and of Koch and Ruhsam have long since been superseded by more modern procedures, and the tables used in connection with them are therefore omitted.

Application of Allihn's Method to the Determination of Other Reducing Sugars. Allihn's method has been employed for determining other reducing sugars besides glucose. Hönig and Jesser[98] have used the method for determining fructose and have constructed a table giving the copper-reducing power of fructose for different weights of sugar. In Table CIX the fructose values of Hönig and Jesser, and the corresponding glucose values of Allihn, are given for several weights of reduced copper. The ratio of fructose to glucose, for the same weight of copper, is also given.

For equal weights of sugar the amount of copper reduced by fructose is about 92 per cent of that reduced by glucose. Soxhlet found by his volumetric method (p. 746) that for equal weights of sugar the reducing power of fructose was 92.4 per cent that of glucose.

Reducing Ratios of Sugars. It is seen from Table CIX that, if the values are eliminated for weights of sugar under 50 mg., for which, as previously stated, Allihn's method gives uncertain results, the ratio of fructose to glucose for the same weight of reduced copper is practically constant and averages 0.915. Other monosaccharides show a

[97] *J. Soc. Chem. Ind.*, **13**, 1227 (1894).

[98] *Monatsh.*, **9**, 562 (1888).

TABLE CIX

Comparative Reducing Power of Fructose and Glucose

Reduced Copper	Fructose (Hönig and Jesser)	Glucose (Allihn)	Ratio $\frac{\text{Glucose}}{\text{Fructose}}$
mg.			
13.7	10	7.9	0.790
32.7	20	17.4	0.870
51.5	30	26.6	0.887
70.2	40	35.9	0.898
88.7	50	45.3	0.906
107.1	60	54.6	0.910
143.2	80	73.0	0.912
178.9	100	91.5	0.915
213.9	120	110.0	0.917
248.3	140	128.3	0.916
282.2	160	146.7	0.917
315.3	180	165.0	0.917
347.9	200	183.1	0.916
379.9	220	201.3	0.915
411.3	240	219.5	0.915
Average ratio (excluding first 5 of the series)			0.915

similar constancy of ratio. The following ratios are given by Browne[99] for four monosaccharides, the copper-reducing power being determined by three different methods:

Reducing Ratios Based on Glucose

Method	Fructose	Galactose	Arabinose	Xylose	Invert Sugar
Allihn's	0.915	0.898	1.032	0.983	0.958
Schoorl's (p. 828)	0.936	0.911	1.012	0.975	.....
Kjeldahl's (p. 798)	0.921	0.915	.....	.....	.....

The reducing ratios of the four sugars calculated for the three different methods do not vary greatly. In calculating the reducing ratios for any particular method, it is necessary, of course, to average all the values obtained at regular intervals and showing fair constancy, but not to average only the two extremes.

If the copper-reducing power of a sugar is determined (as by Allihn's method), the corresponding glucose value of Allihn's table divided by the reducing ratio of the sugar to glucose will give the weight of sugar in the 25 ml. of solution.

Example. Twenty-five milliliters of a fructose solution gave by Allihn's method 265.3 mg. of copper.

[99] *J. Am. Chem. Soc.*, 28, 439 (1906); *Intern. Sugar J.*, 23, 35 (1921).

The amount of glucose corresponding to 265.3 mg. of copper, according to Allihn's table, is 137.45 mg.; 137.45 ÷ 0.915 (the reducing ratio of fructose to glucose) = 150.2 mg. of fructose. The amount of fructose corresponding to 265.3 mg. of copper according to Hönig and Jesser is 150 mg.

The disaccharides lactose and maltose do not show usually the same constancy in reducing ratios for different weights of copper as the monosaccharides. This is due to the partial hydrolysis of the disaccharides as previously explained; the reducing ratio is usually higher the greater the amount of disaccharide. The copper-reducing ratios of lactose and maltose are approximately as follows for Allihn's method:

$$\frac{\text{Glucose}}{\text{Lactose hydrate}} = 0.66 \text{ to } 0.71, \text{ or approximately } 0.7$$

$$\frac{\text{Glucose}}{\text{Maltose}} = 0.56 \text{ to } 0.62, \text{ or approximately } 0.6$$

Quisumbing and Thomas (see p. 802) found somewhat lower reducing ratios by their method, 0.59 to 0.64 or an average of 0.618 for lactose hydrate, and 0.51 to 0.56 or an average of 0.534 for maltose, over a range from 50 to 450 mg. of reduced copper.

Relative Copper-Reducing Power. Instead of the ratios of the weights of sugars for the same amount of reduced copper, the ratios of the weights of copper reduced by the same amount of the two sugars are frequently used. O'Sullivan[100] expressed the relative copper-reducing power of a sugar by the symbol K and adopted as his standard ($K = 100$) the cupric oxide reduced by a given weight of glucose under the conditions of his method. O'Sullivan found, for example, that 1 g. of glucose reduced 2.205 g. cupric oxide and 1 g. of maltose 1.345 g. cupric oxide. The relative copper, or cupric oxide, reducing power of maltose would then be $K = \frac{1.345}{2.205} \times 100 = 61$.

In the examination of starch-conversion products the copper-reducing power of maltose, expressed by the symbol R, is sometimes taken as the standard. Taking the previous values of O'Sullivan, the R of glucose would be $\frac{2.205}{1.345} \times 100 = 164$.

In place of the constant K, Brown, Morris, and Millar[101] have substituted the value κ, which is $\frac{1}{100} K$. According to this system the relative copper-reducing power of maltose, κ (using O'Sullivan's results), is 0.61. The values for κ, when determined for the same absolute weights

[100] *J. Chem. Soc.*, **35**, 770 (1879).
[101] *J. Chem. Soc.*, **71**, 72 (1897).

of the two sugars, are practically identical with the reducing ratios as calculated in the previous section.

Thus, from Defren's table for glucose and maltose, 44.4 mg. of glucose reduces 100 mg. cupric oxide and 44.4 mg. of maltose reduces 61.1 mg. cupric oxide; then $\frac{61.1}{100} = 0.611$, κ for maltose.

Using again Defren's table 44.4 mg. glucose and 72.8 mg. maltose reduce respectively 100 mg. CuO; then $\frac{44.4}{72.8} = 0.610$, the reducing ratio of maltose to glucose.

If κ, however, is calculated from the weights of sugars as determined by the solution factor 3.86, as is sometimes done, then the true reducing ratio is not found unless a correction is applied as indicated on p. 55.

Factors Influencing Reducing Ratios. Later investigations have shown that the reducing ratios may vary considerably, depending on experimental conditions.

1. Effect of reagent used for the determinations. Large variations have been found in the reducing ratios of various sugars with changes in the composition of the reagent used for the determination. With Fehling's solution, for example, considerably more fructose than glucose is required to reduce a given amount of copper; the reverse is true for Ost's copper carbonate reagent (p. 822) and for Steinhoff's copper acetate reagent (p. 820); with Scales's copper citrate reagent (p. 843) the reducing power of the two sugars is about equal.

When the copper reagent contains an optically active constituent, as for instance *d*-tartrate (Rochelle salt in Fehling's and similar solutions), the reducing effect of a sugar belonging to the *d* series is quite different from that of the corresponding *l* sugar. The same difference is noted in the reducing effect of *d* and *l* sugars upon a copper reagent prepared with *l*-tartrate. The *d* sugar has practically the same reducing power toward the *l* reagent as the *l* sugar has toward the *d* reagent; likewise the reducing effect of the *d* sugar on the *d* reagent is about the same as that of the *l* sugar on the *l* reagent.[102]

2. Effect of sugar concentration. In many cases the reducing ratio of monosaccharides is not as constant as shown for fructose when determined by Allihn's method (Table CIX), and it is probable that the gradual rise in the ratio for less than 50 mg. of copper is not due entirely to experimental error. Hammond's table for the Munson and Walker method (Appendix, Table 19B) shows that between 50 and

[102] Richtmyer and Hudson, *J. Am. Chem. Soc.*, 58, 2540 (1936); see also Everett and Sheppard, *J. Am. Chem. Soc.*, 60, 1792 (1938).

320 mg. of reduced copper the reducing ratio of fructose to glucose gradually increases from 0.909 to 0.937.

3. Mutual effect of mixed sugars. When two or more sugars are mixed the reducing ratio of each one is affected by the presence of the others. This subject is discussed more fully in Chapter XVI, in connection with the analysis of sugar mixtures.

Even non-reducing sugars mixed with a reducing sugar affect the reducing power of the latter. This is illustrated by the following figures based on the work of Quisumbing and Thomas (see p. 802) with a modified Fehling's solution:

RELATIVE REDUCING POWER κ

	No Sucrose	2 g. Sucrose
Invert sugar ÷ glucose		
20 mg.	0.927	1.034
200 mg.	0.967	0.976
Fructose ÷ invert sugar		
10 mg.	0.950	0.837
40 mg.	0.950	0.918
80 mg.	0.973	0.946
120 mg.	0.960	0.947
160 mg.	0.968	0.953
200 mg.	0.967	0.962
240 mg.	0.970	0.970

As the ratio of sucrose to reducing sugar decreases, the relative copper-reducing power in the presence of sucrose approaches more and more that found in its absence.

In the analysis of raw cane or beet sugars where the reducing sugars amount to only a small percentage of the sucrose present, the reducing ratios shown on p. 793 must therefore be used with caution, as they may lead to considerable error. In such cases it is advisable for the chemist to determine the reducing ratio with mixtures of known composition, approaching that of the material to be analyzed.[103]

Special copper-reduction methods and tables, similar to those of Allihn, have been established for other reducing sugars. It is impossible to describe all these in detail, and only the following examples are given for invert sugar, maltose, and lactose. The methods are taken from Wein's "Zuckertabellen."

Meissl's[104] Method for Determining Invert Sugar. The Soxhlet formula for Fehling's solution is used; 25 ml. of the copper sulfate

[103] Zerban, Hughes, and Wiley, *J. Assoc. Official Agr. Chem.*, **18**, 118 (1935).

[104] *Z. Ver. deut. Zucker-Ind.*, **29**, 1050 (1879).

solution and 25 ml. of the alkaline tartrate solution are mixed with the sugar solution, which should not contain over 0.245 g. of invert sugar. Enough water is added to make the whole up to 100 ml.; the liquid is heated to boiling and kept at ebullition for exactly 2 minutes. The cuprous oxide is then filtered on asbestos and the reduced copper determined by any of the usual methods. The amounts of invert sugar corresponding to different weights of reduced copper are given in the Appendix in Table 17, which was calculated by Wein from Meissl's reduction factors.

Meissl's method is used in Germany[105] for the analysis of beet molasses containing raffinose and at the same time appreciable quantities of invert sugar (see p. 996). The method is slightly modified in that at the end of the boiling period the solution is at once diluted with 100 ml. of water that has been recently boiled and again cooled to room temperature. For weights of copper of 90 mg. or more Wein's table is employed. If less than 90 mg. copper is obtained, the invert sugar is found from Allihn's table for glucose, which procedure, of course, is erroneous because Allihn's copper reagent is different from Soxhlet's and because the difference in the reducing power of glucose and invert sugar is neglected.

Wein's[106] Method for Determining Maltose. The Soxhlet formula for Fehling's solution is used; 25 ml. of the copper sulfate solution and 25 ml. of the alkaline tartrate solution are mixed and heated to boiling; 25 ml. of the sugar solution, which should not contain over 0.25 g. of maltose, is then added and the liquid boiled for exactly 4 minutes. The cuprous oxide is filtered on asbestos and the reduced copper determined by any of the usual methods. The amounts of maltose corresponding to different weights of reduced copper are given in Wein's "Zuckertabellen."

According to Brown, Morris, and Millar,[107] whose results have been confirmed by Ling and Baker,[108] the table of Wein gives results which are about 5 per cent too low.

Zäch[109] has published a new table, for quantities of maltose up to 0.35 g. in 25 ml. solution, but the procedure is slightly different from that used originally by Wein. After the sugar solution has been added to the boiling Soxhlet solution, the mixture is diluted with 25 ml. of water, again brought to boiling, and boiled for 4 minutes.

[105] "Frühling's Anleitung," 10th ed. by Spengler, p. 289, 1932.
[106] Wein's "Tabellen."
[107] *J. Chem. Soc., Trans.*, **71**, 96 (1897).
[108] *J. Chem. Soc., Trans.*, **71**, 509 (1897).
[109] *Mitt. Lebensm. Hyg.*, **26**, 192 (1935).

Soxhlet's[110] Method for Determining Lactose. The Soxhlet formula for Fehling's solution is used; 25 ml. of the copper sulfate solution and 25 ml. of the alkaline tartrate solution are mixed with 20 to 100 ml. (according to concentration) of the milk-sugar solution, which should not contain over 0.300 g. of lactose hydrate. If less than 100 ml. of milk-sugar solution is taken sufficient water is added to make the whole up to 150 ml. The liquid is then heated to boiling and kept at ebullition for exactly 6 minutes. The cuprous oxide is filtered on asbestos and the reduced copper determined by any of the usual methods. The amounts of lactose hydrate corresponding to different weights of reduced copper are given in Wein's "Zuckertabellen."

The two methods just described are still used to some extent in Europe but have been discarded by the Association of Official Agricultural Chemists. The tables used in connection with them are therefore omitted.

UNIFIED COPPER-REDUCTION METHODS FOR SEVERAL SUGARS

The confusing multiplicity of copper-reducing tables is due to the fact that different investigators have confined their work to one single sugar for one individual set of conditions. A number of chemists, however, have worked with the purpose of establishing one uniform method for all reducing sugars. Examples of such unified methods are those of Kjeldahl and Woy, Defren, Munson and Walker, Bertrand, Lane and Eynon, and others.

Unified Method of Kjeldahl[111] and Woy.[112] In Kjeldahl's method, as modified by Woy, the Fehling's solution is prepared for each analysis with a freshly weighed portion of Rochelle salt. The following solutions are used:

(*A*) 69.278 g. of pure $CuSO_4 \cdot 5\,H_2O$ is dissolved to 1000 ml.
(*B*) 130 g. of pure sodium hydroxide (the amount must be established by titration) is dissolved to 1000 ml.

According to the richness of the sugar solution, 15, 30, or 50 ml. of mixed reagent are made up in an Erlenmeyer flask holding about 150 ml.

For 15 ml. of reagent take 7.5 ml. of *A*, 7.5 ml. of *B* and 2.6 g. Rochelle salt.

[110] *J. prakt. Chem.*, **21**, 266 (1880).
[111] *Neue Z. Rübenzuckerind.*, **37**, 29 (1887).
[112] *Chem. Zentr.* **1897**, [2], 986.

For 30 ml. of reagent take 15.0 ml. of *A*, 15.0 ml. of *B* and 5.2 g. Rochelle salt.
For 50 ml. of reagent take 25.0 ml. of *A*, 25.0 ml. of *B* and 8.65 g. Rochelle salt.

The sugar solution is then added, the total volume of liquid in the flask being always brought to 100 ml. The flask is then plunged in a boiling-water bath and heated for exactly 20 minutes, while leading through the liquid a stream of hydrogen, or of illuminating gas which has been freed of oxygen by passing through a gas washer containing pyrogallic acid and sodium hydroxide solution. The reoxidation of the cuprous oxide by the air is in this way prevented. At the end of the 20 minutes the cuprous oxide is filtered on asbestos, washed, ignited, and weighed as cupric oxide. The amounts of glucose, fructose, invert sugar, lactose hydrate, or maltose corresponding to different weights of cupric oxide or copper are found from a table which was calculated by Woy for the 15-, 30-, and 50-ml. volumes of reagent.

The Kjeldahl-Woy method is one of great exactness, being carried out under rigidly defined conditions. The rather complicated details in preparing the copper reagent and in conducting the reduction have prevented the process from coming into extensive use. Woy's table is therefore omitted.

Unified Method of Brown, Morris, and Millar.[113] In this method, which is adapted from a previous process by O'Sullivan, the Fehling's solution is prepared by dissolving 34.6 g. crystallized copper sulfate, 173 g. Rochelle salts, and 65 g. anhydrous sodium hydroxide to 1000 ml.; 50 ml. of the reagent is placed in a beaker of about 250-ml. capacity and of 7.5-cm. diameter. The beaker is set in a boiling-water bath, and when the solution has acquired the same temperature, the measured volume of sugar solution is added and the whole made up to 100 ml. with boiling distilled water. The beaker is covered with a clock glass, returned to the bath, and heated exactly 12 minutes. The cuprous oxide is filtered in a tube or Gooch crucible and weighed as metallic copper or cupric oxide.

This method is extensively used in Great Britain. The original table of Brown, Morris, and Millar gave the weight of copper and cupric oxide which correspond to the same weight of glucose, fructose, and invert sugar, the order of arrangement being the reverse of that in most tables. Elsdon[114] has recalculated the table in the usual form, and has added columns for maltose and lactose. This table is given in the Appendix, Table 18.

[113] *J. Chem. Soc., Trans.*, **71**, 281 (1897).
[114] *Analyst*, **48**, 435 (1923).

Unified Method of Defren.[115] In Defren's method, which is adapted from O'Sullivan, Soxhlet's formula for Fehling's solution is used; 15 ml. of the copper sulfate solution and 15 ml. of the alkaline tartrate solution are diluted with 50 ml. of water in a 300-ml. Erlenmeyer flask. The flask is then immersed for 5 minutes in a boiling-water bath, when 25 ml. of the sugar solution is quickly run in from a burette. The flask is replaced in the bath and heated for exactly 15 minutes. The cuprous oxide is then filtered on asbestos, washed, ignited, and weighed as cupric oxide. The amounts of glucose, maltose, or lactose corresponding to different weights of cupric oxide are found from a table. Defren's method has been superseded in the United States by that of Munson and Walker.

Unified Method of Munson and Walker.[116] This method is extensively used in the American cane-sugar industry and has replaced the Koch and Ruhsam method formerly employed by the American Leather Chemists' and similar associations. It is one of the official methods of the Association of Official Agricultural Chemists, whose directions are as follows:[117]

Transfer 25 ml. of each of the copper sulfate and alkaline tartrate solutions, Soxhlet's modification, to a 400-ml. beaker of alkali-resistant glass and add 50 ml. of the reducing sugar solution, or, if a smaller volume of sugar solution is used, add water to make the final volume 100 ml. Heat the beaker on an asbestos gauze over a Bunsen burner, regulate the flame so that boiling begins in 4 minutes, and continue the boiling for exactly 2 minutes. (It is important that these directions be strictly observed, and, in order to regulate the burner for this purpose, it is advisable to make preliminary tests, using 50 ml. of the reagent and 50 ml. of water before proceeding with the actual determination.) Keep the beaker covered with a watch glass during the heating. Filter the hot solution at once through an asbestos mat in a porcelain Gooch crucible, using suction. Wash the precipitate of cuprous oxide thoroughly with water at a temperature of about 60° C. and either weigh directly as cuprous oxide or determine the quantity of reduced copper by one of the methods previously described. Conduct a blank determination, using 50 ml. of the reagent and 50 ml. of water, and, if the weight of cuprous oxide obtained exceeds 0.5 mg., correct the result of the reducing sugar determination accordingly. The alkaline tartrate solution deteriorates on standing, and the quantity of cuprous oxide obtained in the blank increases.

Munson and Walker weighed the reduced copper in the form of cuprous oxide. The amounts of glucose, invert sugar, maltose, or lactose

[115] *J. Am. Chem. Soc.*, **18**, 751 (1896).
[116] *J. Am. Chem. Soc.*, **28**, 663 (1906); **29**, 541 (1907); **34**, 202 (1912).
[117] "Methods of Analysis, A. O. A. C.," 5th ed., p. 500, 1940.

corresponding to different weights of cuprous oxide or copper, as found by them, are given in the Appendix, Table 19A.

Jackson[118] has found that, when glucose is determined by the Munson and Walker method, and the reduced copper is measured volumetrically by the modified thiosulfate procedure (p. 779), the amount of copper obtained checks closely with the values given in the table of Munson and Walker, except at very high and very low concentrations of glucose. The relation between milligrams of copper and milligrams of glucose (d) is expressed by the following equation:

$$\text{Cu} = 2.0810\,d - 0.000997\,d^2$$

The results by the volumetric dichromate method (p. 783) also deviate slightly from the figures in the table of Munson and Walker, and satisfy this equation:

$$\text{Cu} = 2.0792\,d - 0.001005\,d^2$$

In 1940 Hammond,[119] at the National Bureau of Standards, revised the Munson and Walker tables for glucose and invert sugar, and added also a column for fructose. In this work sugars of the highest purity were used, and the copper was determined by electrolysis. Hammond's figures are given in the Appendix, Table 19B.

Unified Method of Bertrand.[120] The following formula is used in preparing the copper reagents:

(*A*) 40 g. of pure $CuSO_4 \cdot 5H_2O$ is dissolved to 1000 ml.
(*B*) 200 g. of Rochelle salt and 150 g. of sodium hydroxide in sticks are dissolved to 1000 ml.

Twenty milliliters of the sugar solution, which should not contain over 0.100 g. of reducing sugars, is transferred to a 150-ml. Erlenmeyer flask, and 20 ml. each of solutions A and B added. The liquid is then heated to boiling and kept at gentle ebullition for exactly 3 minutes. The solution is then filtered through asbestos, the precipitate of cuprous oxide washed with distilled water, and the reduced copper determined by the volumetric permanganate method. The cuprous oxide must in this case be dissolved in a saturated solution of ferric sulfate in 20 per cent sulfuric acid because Bertrand's tables are based on this procedure.

Bertrand's method has come into extended use, especially in research work on the chemistry of carbohydrates and in the biochemical field.

[118] *J. Assoc. Official Agr. Chem.*, 18, 172 (1935).
[119] *J. Research Nat. Bur. Standards*, 24, 579 (1940).
[120] *Bull. soc. chim.*, 35, 1285 (1906).

The tables of Bertrand, which gave the weights of reduced copper corresponding to the same weights of different sugars, have been recalculated by Kertész[121] for even increments of copper, as is the usual practice. Kertész's tables for glucose, invert sugar, lactose, maltose, mannose, galactose, sorbose, arabinose, xylose, glucuronic acid, and galacturonic acid are reproduced in condensed form in the Appendix, Table 20.

Unified Method of Quisumbing and Thomas.[122] In this method a modified Soxhlet solution is used; the reduction is carried out at 80° C., and for a period of 30 minutes, in order to avoid the errors due to the effect of varying pressure on the boiling point, and also because at 80° the reducing effect of sucrose is much smaller.

The method has been adopted by the Association of Official Agricultural Chemists for the determination of reducing sugars in plants, and is carried out as follows.[123]

REAGENTS

(*a*) *Copper sulfate solution.* Wash crystals of C.P. $CuSO_4 \cdot 5\ H_2O$ free from dust, etc., with water, dissolve in hot water to make a saturated solution, and filter. Determine the copper electrolytically and dilute the solution so that 25 ml. of it will contain 525 mg. of copper or 41.2 g. of $CuSO_4 \cdot 5\ H_2O$ in 500 ml. of solution.

(*b*) *Alkaline tartrate solution.* Prepare a saturated solution of sodium hydroxide (purified by alcohol) and let stand until the insoluble carbonates and other impurities have settled out several days. Siphon off the clear solution and establish its alkalinity by titration with standard acid. Dissolve 173 g. of highest purity Rochelle salt in water in a 500-ml. graduated flask and add the calculated quantity of sodium hydroxide solution so that 500 ml. of this alkaline tartrate solution will contain exactly 65 g. of sodium hydroxide. Make to the mark with water.

DETERMINATION

Measure exactly 25 ml. each of the copper sulfate and alkaline tartrate solutions into a 400 ml. Pyrex or Bohemian glass beaker, the diameter of which is about 9 cm. Add 50 ml. of sugar solution containing preferably 50–150 mg. of sugar. Cover the beaker with a watch glass and place the beaker in a water bath which is maintained at 80°. After digesting exactly 30 minutes, filter the cuprous oxide by suction through a mat of asbestos in a Gooch crucible. Wash the precipitate with water. Determine the copper by one of the methods previously described.

[121] "Recalculated Tables for the Determination of Reducing Sugars by Bertrand's Method," Geneva, N. Y., 1930; *J. Biol. Chem.*, **108**, 127 (1935).

[122] *J. Am. Chem. Soc.*, **43**, 1503 (1921).

[123] "Methods of Analysis, A. O. A. C.," 5th ed., pp. 138–139, 1940.

The weights of glucose, fructose, invert sugar, lactose, or maltose are found from Table 21 in the Appendix.

Unified Method of Lane and Eynon. The authors of this method (p. 753), employing direct titration of Soxhlet solution with the sugar solution, have applied it not only to glucose, but also to fructose, invert sugar, lactose, and maltose. The corresponding tables are given in the Appendix, Tables 12 and 13.

Methods for Determining Reducing Sugars in the Presence of Sucrose

Reference has been made to the hydrolytic action of hot Fehling's solution upon the higher saccharides. While this action in case of sucrose is slight it is, nevertheless, sufficiently pronounced to cause a considerable error in the determination of reducing sugars when much sucrose is present.

Conditions Affecting the Reducing Action of Sucrose upon Fehling's Solution. The reducing action of sucrose upon Fehling's solution is proportional, first, to the concentration of the sucrose, and second, to the amount of copper left unreduced. If enough reducing sugars are present to precipitate nearly all the copper from the Fehling's solution the hydrolysis of the sucrose is small in extent. This is shown in Table CX, which gives a series of experiments by Browne.[124] The concentrations of sucrose and glucose were varied within wide limits, and determinations of glucose were made by Allihn's method.

The error in the glucose determination when sucrose is present is seen to be considerable; it is even more pronounced in such reduction methods as those of Kjeldahl and Pflüger, which employ a long period of heating.

In a general way the error in the glucose determination is directly proportional to the amount of sucrose, and inversely proportional to the amount of glucose. The correction to be applied is not expressed exactly by the quantity $S \div G$, but by the modification $S \div (G + a)$, in which S represents the milligrams of sucrose by the Clerget method, G the uncorrected milligrams of glucose corresponding to the weight of reduced copper, and a an analytical constant which is unchanged for any given method.

For concentrations of sucrose not exceeding 2000 mg., and of glucose above 50 mg., the value of a is 40, if Allihn's method is used. Curves plotted for a wider range of values, using the formula $S \div (G + 40)$, begin to deviate from actual results when the sucrose approaches very

[124] *J. Am. Chem. Soc.*, **28**, 451 (1906); *J. Assoc. Official Agr. Chem.*, **3**, 261 (1919).

TABLE CX

Correction for the Reducing Action of Sucrose on Allihn's Copper Solution

Taken		Glucose Found (G)	$\frac{S}{G+40}$	Correction (C) $\frac{S}{G+40+\frac{3S^2}{1000G^2}}$	Corrected Glucose ($G-C$)	Error
Sucrose (S)	Glucose					
mg.	mg.	mg.	mg.	mg.	mg.	mg.
250	50.0	52.3	2.7	2.7	49.6	−0.4
250	100.0	102.8	1.8	1.8	101.0	+1.0
250	150.0	151.8	1.3	1.3	150.5	+0.5
250	200.0	199.0	1.0	1.0	198.0	−2.0
500	100.0	104.5	3.5	3.2	101.3	+1.3
500	150.0	153.2	2.6	2.6	150.6	+0.6
500	200.0	203.2	2.1	2.0	201.2	+1.2
500	250.0	251.3	1.7	1.6	249.7	−0.3
1000	50.0	60.3	10.0	9.9	50.4	+0.4
1000	100.0	108.2	6.8	6.7	101.5	+1.5
1000	200.0	205.3	4.1	4.1	201.2	+1.2
1000	250.0	252.0	3.4	3.4	248.6	−1.4
2000	50.0	66.6	18.8	18.3	48.3	−1.7
2000	100.0	113.7	13.0	12.9	100.8	+0.8
2000	200.0	207.5	8.1	8.1	199.4	−0.6
2000	250.0	255.5	6.8	6.7	248.8	−1.2
3000	00.0	26.5	....	28.6	−2.1	−2.1
3000	50.5	75.8	....	24.9	50.9	+0.4
3000	202.2	212.8	....	11.8	201.0	−1.2
5938	62.5	101.0	....	39.2	61.8	−0.7
5625	49.9	91.6	....	41.6	50.0	+0.1
5938	25.6	70.3	....	45.0	25.3	−0.3
6000	00.0	41.7	....	41.8	−0.1	−0.1
6125	11.2	58.2	....	46.5	11.7	+0.5
6125	25.0	69.7	....	46.8	22.9	−2.1
9000	00.0	50.2	....	48.2	2.0	+2.0
12000	00.0	49.0	....	44.6	4.4	+4.4
15000	00.0	50.9	....	42.7	8.2	+8.2

large amounts and reducing sugars very small amounts. Concentrations of sucrose up to 9 g. in 25 ml. show an increase in reducing action; between 9 and 15 g. the reducing action is approximately constant; and with amounts exceeding 15 g. in 25 ml. the action undergoes a decrease.

While it is impossible to establish any simple numerical relationships between the reducing power of sucrose and glucose for all concentrations, it has been found possible to do this algebraically for as high concentrations as are necessary in ordinary analysis.

If it is desired to correct for the retarding influence of high concentrations of sucrose upon the reduction when using Allihn's method, the formula $\frac{S}{G+40}$ is modified to $\frac{S}{G+40+\frac{3\,S^2}{1000\,G^2}}$. The quantity $\frac{3\,S^2}{1000\,G^2}$ is negligible for amounts of sucrose less than 1 g., but with quantities much above 1 g. retardation in its reducing power becomes so pronounced that the additional correction must be made.

To avoid the laborious calculations, Browne recommends the construction of a graph, an example of which is shown in Fig. 287. The

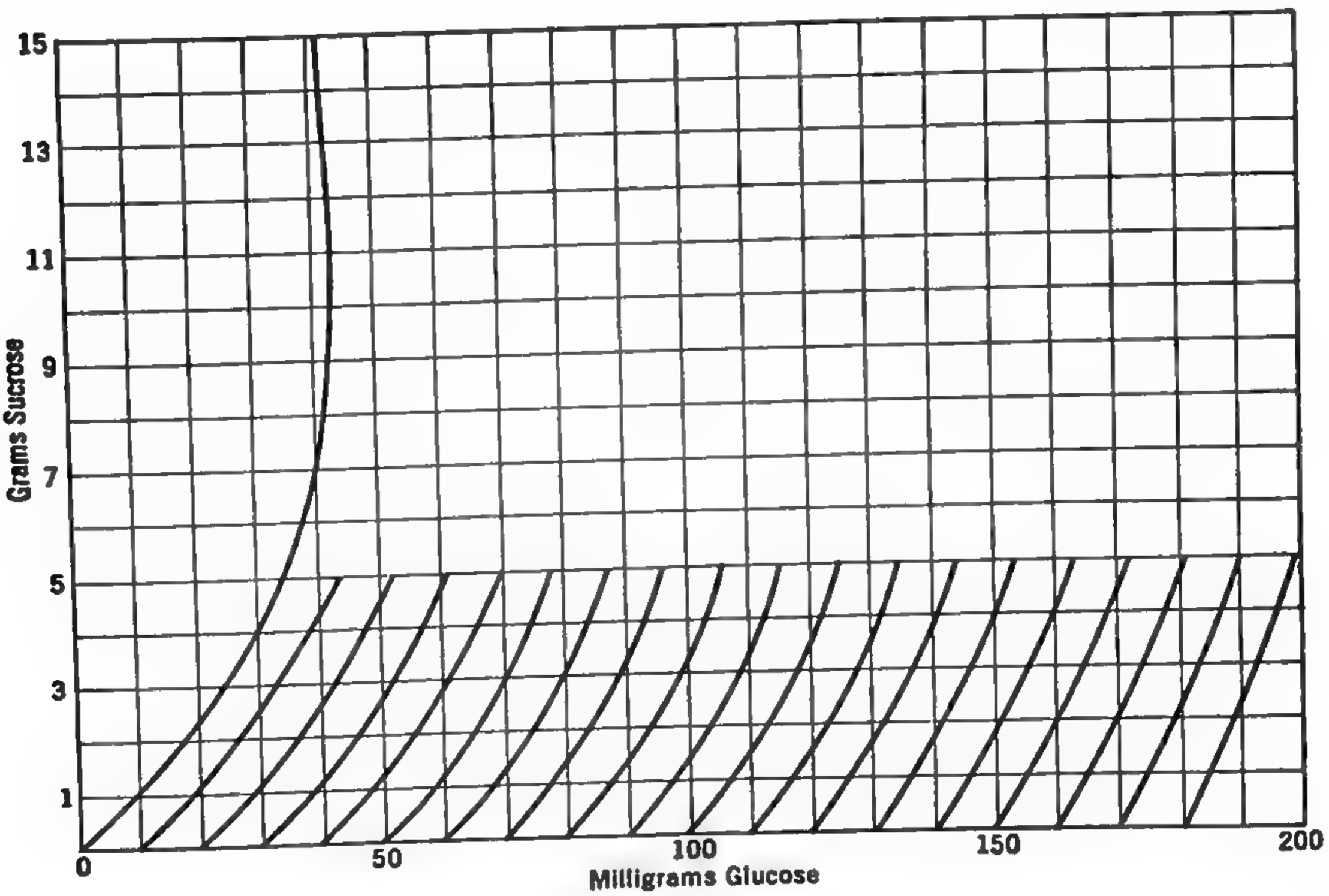


FIG. 287. Browne's graphical procedure for correcting glucose found in the presence of sucrose, by Allihn's method.

grams sucrose are plotted as ordinates, the milligrams glucose as abscissas. A family of curves is drawn in, each of which starts from the base line at the point indicating a given quantity of glucose (10, 20, 30 mg., etc.) at 0 concentration of sucrose and connects it with all the points indicating milligrams of uncorrected glucose at increasing quantities of sucrose. Intermediate amounts of glucose can be readily interpolated. Owing to the change in the character of the curves above

5 g. of sucrose it is not advisable to employ solutions which contain more than this amount of sucrose in 25 ml.

The use of the graph is very simple. Supposing that 80 mg. of glucose is found in the presence of 3 g. of sucrose, follow up the vertical coordinate from 80 mg. at the bottom of the graph until it intersects with the horizontal coordinate from the 3 g. of sucrose, then follow back on the curve which passes through this intersection, and the figure 60 at the bottom indicates the milligrams of glucose actually present.

The falling off in the reducing effect of large amounts of sucrose may be ascribed to its action as a protective colloid, which has been referred to previously (p. 789), and consequent precipitation of the cuprous oxide in such finely divided form that some of it escapes filtration.

Maquenne[125] offered a different explanation, namely, the formation of complex sucrates of copper and sodium or potassium, whose dissociation constants decrease as the sucrose concentration increases. Quisumbing and Thomas[126] arrived at the same conclusion, and this has been further strengthened by the work of Bruhns.[127]

A number of copper-reduction methods have been designed for determining invert sugar in sugar-house products.

Herzfeld's[128] Method for Determining Invert Sugar in Raw Sugars Containing Less than 1.5 Per Cent Invert Sugar. This method is designed for the analysis of the higher grades of raw sugar. The sugar solution, which should contain 20 g. of material in 100 ml. and be free from suspended or soluble impurities, is conveniently prepared as follows:

Dissolve 44 g. of sugar in about 100 ml. of water in a 200-ml. graduated flask. A little neutral lead acetate solution, just sufficient for clarification, is then added and the volume completed to 200 ml. The solution is shaken and filtered, and 100 ml. of the filtrate (22 g. sugar) is measured into a 100–110-ml. flask. A sufficient quantity of a solution of disodium phosphate or potassium oxalate solution is then added to precipitate the excess of lead, and the volume is made up to 110 ml. The solution is shaken, filtered, and 50 ml. of the filtrate (10 g. of sugar) used for the determination.

Or 40 g. of the sugar is dissolved in a 200-ml. flask, clarified with neutral lead acetate solution, the solution made to the mark and

[125] *Compt. rend.*, **161**, 617 (1915).
[126] *J. Am. Chem. Soc.*, **43**, 1503 (1921).
[127] *Centr. Zuckerind.*, **37**, 280, 852, 874, 1268, 1467 (1929).
[128] *Z. Ver. deut. Zucker-Ind.*, **35**, 985 (1885).

filtered. The filtrate is deleaded with dry sodium or potassium oxalate, filtered again, and 50-ml. portions of the final filtrate are taken for analysis. Meade and Harris[129] have shown that in the case of cane-sugar products clarification without lead, using only kieselguhr, gives consistent results; this is a simple method which is used extensively in Cuba. The clarification of sugar-house products is more fully discussed on p. 882.

Transfer 50 ml. of mixed Soxhlet reagent and 50 ml. of the prepared sugar solution to a 250–300-ml. beaker or wide-mouth Erlenmeyer flask, and place it on a piece of asbestos board having a circular opening 6.5 cm. in diameter and resting on a wire gauze. Regulate the burner so that the liquid begins to boil in not less than 3 and not more than 4 minutes. Boil for exactly 2 minutes, and add at once 100 ml. of freshly boiled and cooled distilled water. Filter through asbestos, and determine the copper by one of the methods previously described. The amounts of invert sugar corresponding to different weights of copper are given in the Appendix, Table 22.

Baumann[130] has extended the range of Herzfeld's method to sugars containing as much as 3 per cent invert sugar, by using only 5 g. of raw sugar instead of 10 g. in 50 ml. solution. Otherwise the determination is carried out in exactly the same way. Baumann's table is given in the Appendix (Table 23).

Beet molasses contain only about 50 per cent of sucrose, and the Herzfeld or Baumann tables can therefore not be used to find the invert sugar contained in them. But Schrefeld[131] has established such a table (Table 24 in the Appendix) for a concentration of 5 g. molasses in 50 ml. solution, boiled with 50 ml. mixed Soxhlet solution according to the Herzfeld procedure.

Although Herzfeld's directions are quite explicit and permit little latitude in manipulation, many chemists have found it difficult to check their own results or those obtained by others when the invert-sugar content is very small. This matter is of importance in some European countries where trade rules have set a maximum of 0.05 per cent invert sugar for certain raw beet sugars. The discrepancies may, as in Allihn's method, be ascribed to precipitation of cuprous oxide in colloidal form with the result that some of it escapes filtration. Bruhns[132] made the observation that the cuprous oxide precipitated at the beginning is the highly dispersed yellow modification, and that the

[129] *J. Ind. Eng. Chem.*, **8**, 504 (1916); **13**, 925 (1921).
[130] *Z. Ver. deut. Zucker-Ind.*, **42**, 824 (1892).
[131] *Z. Ver. deut. Zucker-Ind.*, **61**, 982 (1911).
[132] *Centr. Zuckerind.*, **30**, 1473 (1922).

discrepancies in the analytical results disappear when a small quantity of powdered talc is added to the reaction mixture. But he, as well as Pick,[133] Ofner,[134] and Vondrák[135] found that, when finely powdered substances are added, the weight of the cuprous oxide obtained is less, and not more, than when they are not used. The primary cause of the discrepancies is overheating of the strongly alkaline solution. The temperature of boiling varies within wide limits, depending upon the surface of the glass vessel, whether smooth or scratched, and particularly on the nature and quantity of particles suspended in the liquid. With new glass vessels and carefully filtered solutions the boiling point may reach 106–107° C. But when overheating is prevented by the addition of small quantities of powdered talc or wood charcoal, the temperature sinks at once to about 102° C., and the quantity of copper precipitated is as much as 22 mg. lower. Agitation of the flask during the heating period also reduces the boiling temperature. However, cuprous oxide itself does not prevent overheating.

In Germany and in Czechoslovakia the addition of a few glass beads has been recommended[135] to prevent overheating, but if any such deviations from the original Herzfeld procedure are resorted to, Herzfeld's table no longer applies. Even agitation of the flask, which was not used by Herzfeld,[136] must be avoided.

Pick's Modification of the Herzfeld Method.[137] In order to do away with the discrepancies observed for Herzfeld's method, Pick has modified it by adding to the mixture of Soxhlet and sugar solution, before heating, from 25 to 35 mg. of powdered wood charcoal, or 40 to 60 mg. of powdered talc. The mixture is brought to boiling in 3 minutes and boiled for 2 minutes, exactly as directed by Herzfeld. The charcoal is prepared by boiling it out with 20 per cent nitric acid, washing thoroughly with water, and then heating it in a covered porcelain crucible over a blast lamp. The talc powder must first be tested because some varieties are less suitable than others; the temperature of the boiling mixture of Soxhlet and sugar solution must not rise above 103.5° C. When wood charcoal is used, the copper is determined gravimetrically; the trace of ash left in it is too small to affect the result. When talc is employed the copper is determined volumetrically by the permanganate method (p. 776). A comparison of Pick's tables with Herzfeld's table shows that in the gravimetric method the copper

[133] *Z. Zuckerind. čechoslovak. Rep.*, **49**, 211, 219, 235, 243 (1924/25).
[134] *Z. Zuckerind. čechoslovak. Rep.*, **50**, 355 (1925/26).
[135] *Z. Zuckerind. čechoslovak. Rep.*, **58**, 1, 281, 291, 390 (1933/34).
[136] Bruhns, *Z. Zuckerind. čechoslovak. Rep.*, **47**, 373 (1922/23).
[137] *Loc. cit.*

precipitated is from 9 to 18 mg., and in the volumetric method from 14 to 22 mg., lower than in the original Herzfeld method.

Meissl and Wein's[138] Method for Determining Invert Sugar in Mixtures of 90 to 99 Per Cent Sucrose with 10 to 1 Per Cent Invert Sugar. This method is designed for the analysis of low-grade raw sugars, or of other sugar-house products which do not contain a large amount of invert sugar. The sugar solution is prepared as in Herzfeld's method, the final filtrate being diluted if necessary so as not to contain more than 0.2 to 0.245 g. of invert sugar in 50 ml.

Mix 25 ml. each of the copper sulfate and alkaline tartrate solutions (Soxhlet's formula) with the 50 ml. of clarified sugar solution; the liquid is then heated to boiling and kept at gentle ebullition for exactly 2 minutes. After the addition of 100 ml. recently boiled, cold water the cuprous oxide is filtered on asbestos, washed, and the reduced copper determined by any of the usual methods.

For determining the weights of invert sugar corresponding to different weights of reduced copper, for percentages of sucrose between 90 and 99, the following condensed table has been calculated by Wein. Intermediary values can be easily calculated by interpolating.

TABLE CXI

FOR DETERMINING INVERT SUGAR IN THE PRESENCE OF SUCROSE
(Meissl and Wein)

In Mixtures of Sucrose (S) and Invert Sugar (I) in Parts per 100	Milligrams of Invert Sugar								
	245	225	200	175	150	125	100	75	50
	Correspond to Milligrams of Copper								
99 S + 1 I	.....	.....	417.3	370.8	323.6	277.5	230.0	182.0	131.5
98 S + 2 I	.....	.....	393.7	357.7	304.7	259.7	213.7	166.0	113.8
97 S + 3 I	.....	.....	385.7	350.6	298.4	253.8	207.9	158.3	107.9
96 S + 4 I	.....	.....	381.7	339.1	295.3	250.8	205.0	155.4	105.7
95 S + 5 I	439.7	420.1	379.3	337.0	293.4	249.0	203.3	153.6	103.2
94 S + 6 I	438.5	416.5	376.6	334.7	290.1	245.4	199.8	151.0	101.5
93 S + 7 I	437.6	413.9	374.6	332.3	287.8	242.9	197.3	149.2	100.2
92 S + 8 I	437.0	411.9	373.1	330.4	286.3	241.0	195.4	147.9	99.3
91 S + 9 I	436.5	410.3	372.0	328.8	285.1	239.4	193.9	146.8	98.6
90 S + 10 I	436.1	409.2	371.1	327.8	284.0	238.2	192.7	146.0	98.0

The employment of Table CXI is best understood from an example:

A sugar, which indicated 96.2 per cent sucrose by Clerget's method, was made up so that 50 ml. of the clarified and deleaded solution contained 10 g. of sample. The amount of reduced copper obtained by Meissl's method was 324 mg. Required the percentage of invert sugar.

[138] Wein's "Tabellen."

The invert sugar corresponding to 324 mg. copper according to Meissl's table for invert sugar alone is 178 mg. or 1.78 per cent (uncorrected). The percentage composition, in a mixture of 96.2 parts sucrose with 1.78 parts invert sugar, is approximately 98 per cent sucrose and 2 per cent invert sugar. Opposite the mixture 98 $S + 2\ I$ of the table it is seen that

357.7 mg. of copper = 175 mg. invert sugar

and 304.7 mg. of copper = 150 mg. invert sugar

then for the intermediary 324.0 mg. of copper

$$\frac{324.0 - 304.7}{357.7 - 304.7} = 0.36. \quad (175 - 150) \times 0.36 = 9.0 \text{ mg.} \quad 150 + 9.0 = 159.0$$

mg. of invert sugar or 1.59 per cent.

Meissl and Wein's method is not applicable to products which contain more than 10 parts invert sugar in 100 parts of mixed sugars. For this reason the method has largely given place to the more general process of Meissl and Hiller.

Meissl and Hiller's[139] Method for Determining Invert Sugar in Mixtures Containing less than 99 Per Cent Sucrose and More than 1 Per Cent Invert Sugar. This method is designed for the analysis of all sugar-house products except the highest grades of raw sugars. The method is based upon the principle of taking such a quantity of material for analysis that the invert sugar will reduce nearly all the copper, thus reducing the error due to presence of sucrose to a minimum.

The sugar solution is prepared as in the two previous methods so that 100 ml., after clarification and deleading, contains 20 g. of sample. Prepare a series of solutions in large test tubes by adding 1, 2, 3, 4, and 5 ml. of this solution to each tube successively. Add 5 ml. of the mixed copper reagent (Soxhlet's formula) to each, heat to boiling 2 minutes, and filter. Note the volume of sugar solution which gives the filtrate lightest in tint, but still distinctly blue. Place 20 times this volume of the sugar solution in a 100-ml. flask, dilute to the mark, and mix well. Use 50 ml. of the solution for the determination, which is conducted as in the method of Meissl and Wein. The invert sugar is then calculated by means of the following formulas.

Let Cu = the weight of copper obtained.

P = the polarization of the sample.

W = the weight of sample in the 50 ml. of solution used for determination.

[139] *Z. Ver. deut. Zucker-Ind.*, **39**, 735 (1889).

F = the factor obtained from the table for conversion of copper to invert sugar.

$\frac{Cu}{2}$ = approximate weight of invert sugar = A.

$A \times \frac{100}{W}$ = approximate percentage of invert sugar = y.

$\frac{100\,P}{P + y}$ = S, approximate percentage of sucrose in mixture of sugars.

$100 - S = I$, approximate percentage of invert sugar.

$\frac{Cu\,F}{W}$ = percentage of invert sugar.

The factor F for calculating copper to invert sugar is then found from Table CXII.

TABLE CXII

MEISSL AND HILLER'S FACTORS FOR CALCULATING COPPER TO INVERT SUGAR FOR DIFFERENT RATIOS OF SUCROSE TO INVERT SUGAR

Ratio of Sucrose to Invert Sugar = $S : I$	Approximate Weight of Invert Sugar = A						
	200 mg.	175 mg.	150 mg.	125 mg.	100 mg.	75 mg.	50 mg.
0 : 100	56.4	55.4	54.5	53.8	53.2	53.0	53.0
10 : 90	56.3	55.3	54.4	53.8	53.2	52.9	52.9
20 : 80	56.2	55.2	54.3	53.7	53.2	52.7	52.7
30 : 70	56.1	55.1	54.2	53.7	53.2	52.6	52.6
40 : 60	55.9	55.0	54.1	53.6	53.1	52.5	52.4
50 : 50	55.7	54.9	54.0	53.5	53.1	52.3	52.2
60 : 40	55.6	54.7	53.8	53.2	52.8	52.1	51.9
70 : 30	55.5	54.5	53.5	52.9	52.5	51.9	51.6
80 : 20	55.4	54.3	53.3	52.7	52.2	51.7	51.3
90 : 10	54.6	53.6	53.1	52.6	52.1	51.6	51.2
91 : 9	54.1	53.6	52.6	52.1	51.6	51.2	50.7
92 : 8	53.6	53.1	52.1	51.6	51.2	50.7	50.3
93 : 7	53.6	53.1	52.1	51.2	50.7	50.3	49.8
94 : 6	53.1	52.6	51.6	50.7	50.3	49.8	48.9
95 : 5	52.6	52.1	51.2	50.3	49.4	48.9	48.5
96 : 4	52.1	51.2	50.7	49.8	48.9	47.7	46.9
97 : 3	50.7	50.3	49.8	48.9	47.7	46.2	45.1
98 : 2	49.9	48.9	48.5	47.3	45.8	43.3	40.0
99 : 1	47.7	47.3	46.5	45.1	43.3	41.2	38.1

The use of Meissl and Hiller's formulas and table for calculating invert sugar is best understood from an example.

The polarization of a sugar was 86.4; 50 ml. of a solution containing 3.256 g. of sample, reduced by Meissl and Hiller's method, 0.290 g. of copper.

Required the percentage of invert sugar.

$$\frac{\text{Cu}}{2} = \frac{0.290}{2} = 0.145 = A$$

$$A \times \frac{100}{W} = 0.145 \times \frac{100}{3.256} = 4.45 = y$$

$$\frac{100\,P}{P + y} = \frac{8640}{86.4 + 4.45} = 95.1 = S$$

$$100 - S = 100 - 95.1 = I = 4.9$$

$$S : I = 95.1 : 4.9$$

By consulting the table it is seen that the vertical column headed 150 is nearest to A, 145, and the horizontal column having the ratio 95 : 5 is nearest to the ratio of S to I, 95.1 : 4.9. At the intersection of these columns is found the factor 51.2 which enters into the final calculation $\frac{\text{Cu}\,F}{W} = \frac{0.290 \times 51.2}{3.256} =$ 4.56 per cent of invert sugar.

In practical sugar-house control these calculations would require too much time. Rice,[140] Wedderburn, and others[141] have calculated expanded tables for definite quantities of cane juice, sirup, and molasses used for analysis.

Munson and Walker's[142] Method for Determining Invert Sugar in the Presence of Sucrose. Munson and Walker have included in their unified method for reducing sugars determinations of invert sugar in the presence of variable amounts of sucrose. Their table (Appendix, Table 19A) gives the weight of invert sugar for different weights of cuprous oxide or copper, when the total weight of invert sugar and sucrose in the solution taken is 0.4 g. and 2.0 g. The 0.4-g. amount is used preferably for sugar products containing between 1 and 9 parts of sucrose to 1 part of invert sugar and the 2.0-g. amount for sugar products containing over 9 parts of sucrose to 1 part of invert sugar. This range is sufficient to include most of the products of the sugar factory.

The method requires a preliminary investigation of the material in order to determine the approximate percentages of sucrose and invert sugar for use in making up the solution.

Partly inverted sugar sirups and so-called "high test" or inverted molasses frequently contain 3 or more parts of invert sugar to 1 part of sucrose. Supposing that such a product has 25 per cent of sucrose

[140] *8th Intern. Congr. Applied Chem.*, **8**, 47 (1912).

[141] Spencer-Meade, "Handbook for Cane-Sugar Manufacturers," 7th ed., pp. 512–518, 1930.

[142] *J. Am. Chem. Soc.*, **28**, 663 (1906).

and 50 per cent of invert sugar, it would be necessary to prepare a solution containing 0.4 ÷ 0.75, or 0.533 g., in 50 ml. But this solution would have 0.267 g. invert sugar, which is beyond the range of the Munson and Walker table. This difficulty can be overcome by adding dry, pure sucrose, containing only traces of invert sugar, in preparing the solution. For example, 4 g. of the product and 1 g. sucrose are made up, after clarification, to 500 ml. total volume. This solution will then contain, in 50 ml., 0.3 plus 0.1, or 0.4 g. total sugars and 0.2 g. invert sugar, which is within the limits of the Munson and Walker table.

The Munson and Walker table published in 1940 by Hammond (see p. 801; Appendix, Table 19B) gives not only the revised values for mixtures of invert sugar and sucrose totaling 0.4 or 2 g., respectively, in 50 ml. solution, but also a new column for 0.3 g. total sugars. This makes it possible to analyze high-test molasses and similar products without the addition of sucrose to the sample.

Determination of Lactose in Milk Chocolate. A polarimetric method for the determination of sucrose and lactose in milk chocolate has been described on p. 453. Fitelson[143] found that the lactose can be estimated more accurately by applying the method of Munson and Walker to an aliquot (20 ml. diluted to 50 ml.) of the clarified and deleaded filtrate used for the polarizations, and titrating the reduced copper by the volumetric thiosulfate procedure (p. 779). The equivalent cuprous oxide found is then corrected for the reducing effect of the sucrose in the following manner. The approximate percentage of lactose is obtained from the direct polarization P and the sucrose S by this formula:

$$\text{Approximate percentage of lactose} = \frac{P(1.1 + 0.01\,X) - S}{0.79}$$

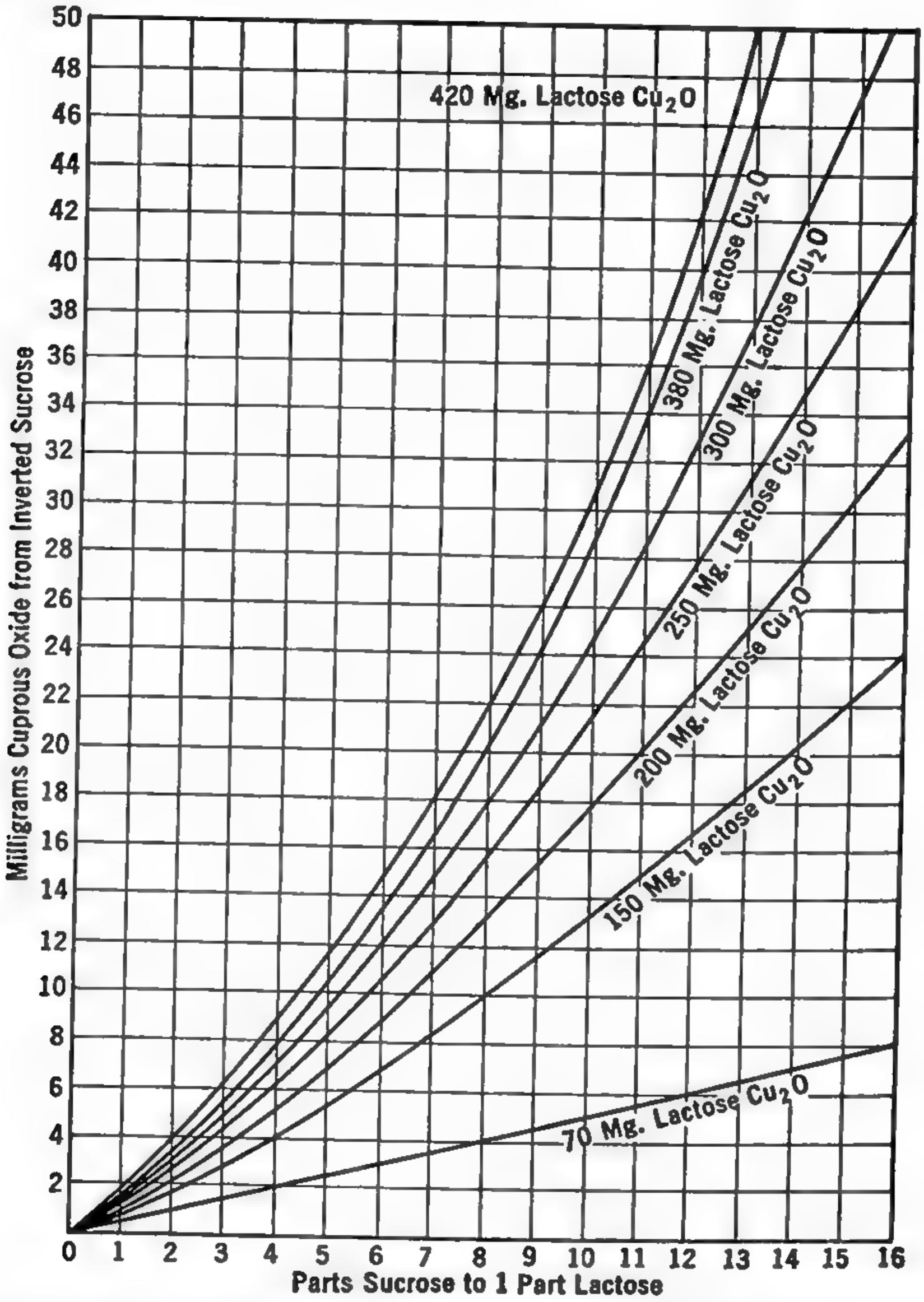
in which X has the value given on p. 454. The ratio of sucrose to approximate lactose is calculated, and the milligrams of cuprous oxide to be deducted from those found are taken from Fig. 288. The corrected milligrams of cuprous oxide are converted into milligrams of lactose L by the table of Munson and Walker (Appendix, Table 19A), and the corrected percentage of lactose is found by the formula

$$\text{Corrected percentage of lactose} = \frac{L(110 + X)}{0.26\,C}$$

where X has the same value as above, and C is the milliliters of solution used for the determination by the Munson and Walker method (20 ml.).

[143] *J. Assoc. Official Agr. Chem.*, 15, 551 (1932); 16, 564 (1933); 17, 337 (1934); "Methods of Analysis, A. O. A. C.," 5th ed., p. 202, 1940.

A similar method for determining lactose in milk chocolate by means of Fehling solution is given by von Fellenberg.[144]



(Reproduced with permission from "Methods of Analysis, A.O.A.C.," 6th ed., p. 203.)

FIG. 288. Graph used in correcting cuprous oxide for effect of sucrose.

Saillard's Method for Determining Invert Sugar in Beet-Sugar Products.[145] In order to minimize the reducing effect of the sucrose, Saillard does not boil the mixture of sugar solution and copper reagent, but operates at a temperature of 62–64° C. The copper reagent used by him is prepared like Soxhlet's solution, but with 65 g. instead of 50

[144] *Mitt. Lebensm. Hyg.*, **28, 73 (1937).**
[145] *Compt. rend.*, **161, 591 (1915).**

g. of sodium hydroxide. The approximate French normal weight, 16.3 g., of the product to be analyzed is dissolved in a 100-ml. flask, clarified with neutral lead acetate solution, made to the mark, filtered, deleaded with dry sodium carbonate, and refiltered. Twenty milliliters of the mixed copper reagent and 50 ml. of the sugar solution are mixed in a 150-ml. Erlenmeyer, and the flask is placed for exactly 22 minutes in a water bath heated to 62–64° C. The level of the bath should be slightly above that of the liquid in the flask. The flask is shaken from time to time while in the bath. At the end of the heating period the solution is at once filtered through asbestos, the precipitate is washed with warm water, and the copper is determined by the permanganate method according to Bertrand's procedure (p. 801). A solution containing 5 g. of chemically pure potassium permanganate per liter is used for the titration. Saillard's table, showing the milligrams copper (1 ml. permanganate solution = 10 mg. copper) corresponding to various percentages of invert sugar, in the presence of different quantities of sucrose, is given in the Appendix (Table 25).

Method of Pellet-Babinski for Determining Invert Sugar in Raw and Refined Beet Sugars.[146] This method, which is official in Poland follows Saillard's procedure of carrying out the reduction at a temperature below boiling, but the copper reagent is somewhat different Solution A contains 34.64 g. of crystallized copper sulfate in 500 ml. solution B is prepared by dissolving 180 g. Rochelle salt and 60 g. sodium hydroxide to 500 ml. Twenty milliliters of solution, containing 5 g. of the sugar to be analyzed, is mixed with 10 ml. each of solution A, solution B, and of water, in a beaker holding about 140 ml. (45 mm. diameter by 90 mm. high). The total volume of the reaction mixture must always be 50 ml. The beaker is placed in a water bath previously heated to 71° C., and the temperature is so regulated that the liquid in the beaker reaches 61° C. in 3 to 4 minutes. The temperature is then maintained between 61° and 62° C. for 10 minutes longer. The beaker is removed from the bath and 50 ml. of cold distilled water is added. The solution is then filtered twice through asbestos, to collect all the finely divided cuprous oxide. The copper is determined by Bertrand's method (p. 801), being titrated with a solution containing 2.486 g. potassium permanganate per liter, each milliliter of which corresponds to 5 mg. of copper. Babinski found a straight-line relationship between copper and invert sugar. The milligrams of metallic copper found, multiplied by 0.615, gives the milligrams invert sugar in 5 g. of the sugar.

[146] Private communication from M. Werkenthin.

Method of Edwards and Osborn for Determining Invert Sugar in Beet Products. Edwards and Osborn[147] have adapted the Quisumbing and Thomas method (p. 802) to the determination of small percentages of invert sugar in the presence of large amounts of sucrose, on the same principle as that used by Saillard, with a choice between two different procedures. In one of these the reduction is carried out at 80° C., as specified by Quisumbing and Thomas; in the other the observation of Bruhns has been made use of that, if the reaction mixture is heated just to the boiling point, the maximum reducing effect of the invert sugar has been nearly reached, while that of the sucrose is very small, giving only 3.8 mg. copper for 5 g. sucrose.

The copper reagent is prepared as described by Quisumbing and Thomas. The sugar solution is made up in such a way that 50 ml. of the final filtrate contains either 5 or 2.5 g. of dry substance. The number of grams of material, represented by the fraction of 2200 (or 1100) divided by the Brix of the material, is dissolved in hot water and transferred to a 200-ml. flask, the solution cooled, clarified with 10 ml. of neutral lead acetate solution of 55° Brix (422.5 g. crystallized salt per liter), made to the mark, and filtered. Delead 100 ml. of the filtrate with 10 ml. of a solution containing 5 g. sodium oxalate and 5 g. ammonium dihydrogen phosphate in 100 ml., shake, and filter. Place 25 ml. each of solution A and B of the copper reagent in a 250-ml. Erlenmeyer and add 50 ml. of the clarified and deleaded sugar solution. Then proceed according to either of the following methods.

Method I. Cover the flask with a small watch glass and place it for exactly 30 minutes in a water bath kept at 80° C. The level of the liquid in the flask should be 2 inches below the level of the bath.

Method II. Place the Erlenmeyer flask on an electric hot plate which is regulated so that the liquid will reach the boiling point in 2 minutes, with a tolerance of ±10 seconds. As soon as the solution comes to a full boil remove the flask from the heater and immediately add 100 ml. of cold water, which has been boiled previously.

When the reduction by either of these methods is completed, the solution is at once filtered through an asbestos mat about 8 mm. thick, and the precipitate washed, first by decantation and then on the filter. The copper is determined in the precipitate by any convenient method, preferably by Low's volumetric procedure (p. 779). If the titration is carried out in the flask used for the reduction, the precipitate need not be transferred quantitatively to the Gooch crucible.

The tables of Edwards and Osborn are given in the Appendix, Table 26. Parts I to III of the tables are used in connection with Method I,

[147] *Ind. Eng. Chem., Anal. Ed.*, **5**, 42 (1933).

parts IV to VI with Method II. Parts I and IV are for sugars and high-purity juices; parts II and V for molasses of about 60 purity. Parts III and VI are used when the molasses solution was made with 2.5 instead of 5 g. dry substance. The tables may be interpolated for products of varying purity, and to some extent also extrapolated by means of the formulas given at the end of each table.

The basic data for Method II were determined at an altitude of 3930 feet above sea level, where water boils at 96° C. If the method is to be used at other altitudes, the chemist should first check parts IV to VI of the table under his own experimental conditions.

When the copper reagent is first prepared, and again after it has been standing for some time, it should be tested with a solution containing 3 g. pure sucrose in 50 ml. The amount of copper precipitated should be 18.0 mg. by Method I, and 2.8 mg. by Method II. If the results deviate from these values by more than 3 or 4 mg., the cause of the discrepancy must be found and the error corrected.

In the volumetric methods of Soxhlet, Violette, etc., where the invert-sugar solution is added to the point of complete reduction, and no excess of copper is left in solution, the error due to the effect of the sucrose is reduced, but not entirely eliminated, as has been shown by Lane and Eynon.

Lane and Eynon's Volumetric Method for Determining Invert Sugar, Lactose, or Glucose in the Presence of Sucrose. The method is carried out as described previously (p. 753). Lane and Eynon have compiled tables of factors for solutions containing, in 100 ml., up to 25 g. of sucrose in addition to the invert sugar, for titration against 10 ml. Soxhlet solution; or 1 g. of sucrose in addition to the invert sugar, for titration against 25 ml. Soxhlet solution (see Appendix, Tables 12, 12A, and 13). If the amount of invert sugar is less than 0.3 per cent of the sucrose the result falls outside the range of the Lane and Eynon tables. In this case Eynon and Lane[148] recommend the addition of 100 mg. invert sugar, in the form of standard invert-sugar solution, to 25 g. of raw sugar in a 100-ml. flask, before making up to the mark. The solution is titrated against 10 ml. of Soxhlet solution, and the added 100 mg. of invert sugar is deducted from the milligrams of invert sugar found.

The reducing power of lactose and of glucose is also affected by sucrose simultaneously present, and in the analysis of condensed sweetened milk and of chocolate this factor must be considered. For the former, Lane and Eynon[149] have given tables of corrections to be ap-

[148] *J. Soc. Chem. Ind.*, **50**, 85T (1931).
[149] *J. Soc. Chem. Ind.*, **46**, 434 (1927).

plied to mixtures containing sucrose and lactose in the ratio of 3 to 1, and 6 to 1. Corrections for ratios of 12 to 1, 15 to 1, and 20 to 1 have been determined by Fitelson.[150] The same author has also established corrections for mixtures of sucrose and glucose in the ratio of 2 to 1, 4 to 1, 8 to 1, and 20 to 1, to be used in the analysis of sweet chocolate containing both sugars. These corrections, reproduced in Table 27 in the Appendix, are added to the burette readings actually obtained, and the factors corresponding to the corrected readings are found from the original tables of Lane and Eynon for lactose or glucose alone.

Main's Pot Method for Determining Invert Sugar in the Presence of Sucrose. Main's method, described in detail on p. 757, has been designed particularly for the determination of invert sugar in refinery products. One table given by him (Appendix, Table 14), shows the grams (0.143 to 0.648), or the percentage (0.312 to 16.6) of invert sugar, corresponding to 15 to 35 ml. of sugar solution required for complete reduction, for mixtures containing 1, 2.5, 5, or 10 g. of sucrose in 100 ml. solution, and for varying amounts of Soxhlet solution used.

For the determination of very small amounts of invert sugar, down to 0.001 per cent, in the presence of large quantities of sucrose, Main recommends a modified Soxhlet solution, which is made up as follows:

Solution I: 34.639 g. copper sulfate dissolved to 500 ml.
Solution II: 173 g. Rochelle salt, 50 g. sodium hydroxide, and 14.647 g. potassium ferrocyanide dissolved to 500 ml.
Solution III: 5 *N* sodium hydroxide solution (200 g. pure NaOH dissolved to 1 liter).

Immediately before use 1 volume of solution I is mixed with 1 volume of solution II and 2 volumes of solution III. The potassium ferrocyanide converts the cuprous oxide into white cuprous ferrocyanide, and this makes it easier to observe the complete decolorization of the methylene blue indicator. The additional sodium hydroxide hastens the reducing action of the invert sugar on the Soxhlet solution. The "extra alkaline" Soxhlet solution is standardized by mixing 4 ml. of it in Main's special test tubes with varying quantities of standard solution containing 0.025 g. invert sugar in 100 ml., but no sucrose, and adding 2 drops of methylene blue solution. Complete decolorization of the indicator should be effected by 37 ml. of the standard invert-sugar solution upon heating in the boiling-water bath for exactly 5 minutes. The actual determinations of invert sugar are carried out according to the directions given in Table 15 in the Appendix, specify-

[150] *J. Assoc. Official Agr. Chem.*, **15**, 624 (1932).

ing the amounts of sucrose in 100 ml., the quantities of extra alkaline Soxhlet solution to be used, and the time of heating necessary. For samples containing less than 0.01 per cent of invert sugar the heating period is extended to 10 minutes.

Main's method makes it possible to estimate invert sugar in the highest grades of refined cane or beet sugar. It requires more time and manipulation than the Lane and Eynon method, but it gives more accurate results because there is less latitude in its operation. When the approximate percentage of invert sugar in products of high purity is not known considerable time may be saved by a systematic preliminary test. A solution containing 50 g. of the sugar in 250 ml. is prepared, and five tubes are made up as follows:

Tube No.	Sugar Solution	Water	Extra Alkaline Copper Reagent	Methylene Blue Solution
	ml.	ml.	ml.	drops
1	4	12	4	2
2	8	8	4	2
3	16	..	4	2
4	16	..	2	2
5	16	..	1	2

The tubes are heated in the water bath for exactly 5 minutes, removed at once, and examined.

If no tube is blue, the invert sugar is more than 0.80%
" tube 1 " " is 0.37 to 0.80%
" " 2 " " " 0.18 to 0.36%
" " 3 " " " 0.09 to 0.17%
" " 4 " " " 0.06 to 0.08%

If all tubes are still blue the heating is continued for another 5 minutes. If the last tube is decolorized the invert sugar is between 0.01 and 0.04 per cent; if all are still blue, it is less than 0.01 per cent.

Choice of Method for Determining Invert Sugar in Raw Cane Sugars. Zerban, Hughes, and Wiley[151] have made a comparison among the four methods adopted for this purpose by the Association of Official Agricultural Chemists — those of Herzfeld (p. 806), Munson and Walker (p. 812), Allihn-Browne (p. 803), and Lane and Eynon (p. 817). Mixtures of pure sugars as well as raw cane sugars were used in the tests. The Herzfeld method, which has been shown to be unsatisfactory for beet sugars (p. 807), gives reliable results with raw cane sugars, which usually contain above 0.25 per cent of invert sugar. The re-

[151] *J. Assoc. Official Agr. Chem.*, **18**, 118 (1935).

sults found by the method of Lane and Eynon check closely with those of Herzfeld's method, but the values obtained with the Munson and Walker method tend to be too high, and those of the method of Allihn-Browne too low. The Herzfeld or the Lane and Eynon method may therefore be recommended for the determination of invert sugar in raw cane sugars.

Miscellaneous Copper-Reduction Methods

The large amount of free alkali in Fehling's copper solution has proved its most objectionable feature, owing to the influence which it has in rendering sucrose and other substances slightly copper reducing. Attempts have accordingly been made to devise a copper reagent for sugar analysis which would contain no caustic alkali. Although none of the solutions thus designed has shown the same all-around suitability as that of Fehling, a few of them have found a certain usefulness in special cases. In these modified reagents the caustic alkali of Fehling's solution has been replaced by acetate, bicarbonate, carbonate, phosphate, or similar buffer salts.

Barfoed's[152] Copper Acetate Method. Barfoed's copper acetate solution (p. 648), which is not reduced to any great extent by the disaccharides maltose and lactose, has appealed to chemists as a convenient means of determining glucose, fructose, and other monosaccharides in the presence of the higher reducing sugars. But notwithstanding its value for qualitative purposes, attempts to use Barfoed's reagent for the quantitative determination of glucose and other monosaccharides have always given unsatisfactory results.

The principal reason for the difficulties encountered is that the acetic acid is partly volatilized during the heating period and that the oxidizing effect of the reagent varies accordingly. Steinhoff[153] has improved the Barfoed reagent by using a mixture of 1 volume of Soxhlet solution *A* (34.639 g. copper sulfate in 500 ml. solution) with 2 volumes of a solution containing 250 g. sodium acetate in 500 ml. Since this reagent finds its special application in the analysis of sugar mixtures, especially of commercial glucose, but offers no particular advantages in the determination of monosaccharides alone, the method of Steinhoff is described in Chapter XVI.

Soldaini's[154] Copper Bicarbonate Method. Soldaini's copper bicarbonate solution (p. 648) has also appealed to chemists as a means of

[152] *Z. analyt. Chem.*, **12, 27 (1873).**
[153] *Z. Spiritusind.*, **56, 64 (1933).**
[154] *Ber.*, **9, 1126 (1876).**

avoiding certain errors resulting from the use of Fehling's solution. Soldaini's method, however, has usually given unreliable results when used for quantitative purposes, the principal objections being the deposition of copper hydroxide and the precipitation of lime and other mineral impurities with the reduced copper.

Determination of Invert Sugar in Refined Sugar According to Bates and Jackson.[155] These authors have used a modified Soldaini solution for this particular purpose. To prepare the copper reagent, 300 g. of potassium bicarbonate and 1 g. of crystallized copper sulfate are dissolved to a total volume of 1 liter. Ten grams of the sugar is dissolved in water and the solution made up to 50 ml. This is poured into 50 ml. of the copper reagent, and the remainder of the sugar solution transferred with 10 ml. of water. The mixture is heated to boiling, and the boiling continued for exactly 2 minutes. The reaction is stopped by the addition of 100 ml. of cold, recently boiled water. The cuprous oxide precipitate is obtained in very finely divided form, but it can be collected quantitatively on a tight asbestos filter, or better in a Neubauer crucible with platinum sponge. It is weighed as cuprous oxide. Under these conditions sucrose free from invert sugar gives 1.1 mg. cuprous oxide; each added 0.01 per cent of invert sugar reduces an additional 1.9 mg. cuprous oxide. If the milligrams of cuprous oxide found for a given sugar equal a, then the percentage of invert sugar $= 0.01 \times (a - 1.1)/1.9$.

Effect of Composition of the Copper Reagent on the Reducing Power of Invert Sugar and of Sucrose. Spengler, Tödt, and Scheuer[156] have found that the reducing effect of invert sugar and of sucrose depends primarily on the *p*H and the copper concentration of the solution, and secondarily on the temperature and duration of the heating.

Effect of *p*H. When the mixture of copper reagent and sugar solution is heated for varying time periods in a boiling-water bath under reflux, higher *p*H causes an increase in the amount of copper reduced by 10 g. of sucrose for any given time of heating, and an increase in the rate of the reduction. At the same *p*H the amount of copper reduced first increases per unit of time, but later the reaction rate becomes constant. At *p*H 10, ten grams of pure sucrose reduces the same amount of copper as 2 mg. of invert sugar, and smaller quantities of sucrose a proportionately smaller amount.

Invert sugar behaves very differently from sucrose. The quantity of copper reduced rises very rapidly with the time of heating and then

[155] *Bull. Bur. Standards*, 13, 67 (1916); *Sci. Paper* 268.
[156] *Z. Ver. deut. Zucker-Ind.*, 86, 130, 322 (1936).

becomes practically constant after reaching a maximum. The increase in the rate is the higher, the lower the *p*H of the copper reagent. The maximum reduction is also the higher, the lower the *p*H. If the caustic soda in Fehling's solution is replaced by an equivalent quantity of sodium carbonate (Müller's solution), resulting in a *p*H of 10.4, one equivalent of either glucose or fructose reduces exactly six equivalents of copper, over a limited range. The stoichiometric relationship is ascribed by Spengler, Tödt, and Scheuer to the oxidation of both glucose and fructose to arabonic acid.

Effect of Copper Concentration. With Müller's solution an increase in the copper concentration increases the amount of copper reduced by a given quantity of invert sugar, but has little effect on that reduced by sucrose. But with Fehling's solution it increases the amount of copper reduced by both invert sugar and sucrose. Müller's solution is therefore particularly suitable for the determination of small quantities of invert sugar in high-grade sugars. The differences in the behavior of Fehling's and of Müller's solution upon increasing the copper concentration are explained by the change in *p*H when copper sulfate is added to Müller's solution, while with Fehling's solution the *p*H is little affected thereby. All the observations recorded by Spengler, Tödt, and Scheuer indicate that the reaction between copper solutions and reducing sugars is regulated by oxidation-reduction potentials.

Stare[157] has also studied the effect of the composition of the copper reagent on the reducing power of invert sugar and of sucrose, with similar results.

Spengler, Tödt, and Scheuer have based a method for the determination of invert sugar in refined sugars and in beet raw sugars on their observations with Müller's solution. The reduced copper is determined iodometrically without filtration of the mixture, and the method is therefore described, with other methods of this type, on p. 840.

Ost's[158] Copper Bicarbonate Method. Ost has modified Soldaini's reagent in order to eliminate its objectionable features. In his final improvement of the method the copper reagent is prepared as follows: 250 g. of chemically pure potassium carbonate and 100 g. of chemically pure potassium bicarbonate are dissolved in water, and a solution containing 17.5 g. of chemically pure crystallized copper sulfate is slowly added. The volume is then made up to 1000 ml. and the solution filtered through asbestos, the first runnings of the filtrate being rejected.

[157] *Bull. assoc. chim.*, **53**, 456 (1936).
[158] *Chem. Ztg.*, **19**, 1784, 1829 (1895).

In making the determination 100 ml. of the copper reagent is treated with 50 ml. of the sugar solution and the liquid boiled for 10 minutes. The precipitate is then filtered upon asbestos and the reduced copper determined by any of the usual methods.

Ost has unified his method for a number of reducing sugars; a few of the values for different weights of reduced copper are given in Table CXIII.

TABLE CXIII

REDUCING POWER OF DIFFERENT SUGARS UPON OST'S COPPER SOLUTION

Reduced Copper	Glucose	Fructose	Invert Sugar	Maltose
mg.	mg.	mg.	mg.	mg.
100	30.7	29.0	30.0	57.9
150	45.4	42.7	44.4	85.4
200	60.7	57.0	59.0	112.9
250	76.5	71.6	74.3	141.1
300	93.0	87.5	90.9	170.3
350	112.8	106.4	109.8	201.5
400	134.9	128.2	131.0	235.6

The method has not been found to give good results with lactose. Glucose, by Ost's process, reduces about 60 per cent more copper than by Allihn's method.

For determining small amounts of reducing sugars Ost recommends the use of his 0.2 *N* copper solution which contains 250 g. chemically pure potassium carbonate, 100 g. chemically pure potassium bicarbonate, and 3.6 g. chemically pure crystallized copper sulfate to the liter. In using this solution, which is very sensitive towards small amounts of reducing sugars, the time of boiling is reduced to 5 minutes.

Ost's method has given good results in the analysis of pure sugar solutions, but has proved less reliable in the examination of low-grade products owing to the precipitation of lime and other mineral impurities. This difficulty, according to Ost, may be obviated by precipitating the lime with ammonium oxalate during the clarification. The method has not come into use as a general procedure for determining reducing sugars, but it offers certain advantages in some specific problems, such as the determination of invert sugar in the presence of sucrose, and the estimation of fructose in mixtures with other sugars.

Beyersdorfer's[159] Method for Determining Invert Sugar in the Presence of Sucrose. Table CXIII shows that 1 mg. invert sugar reduces over 3 mg. of copper from Ost's solution, while with Soxhlet's solution it gives only about 2 mg. Beyersdorfer found that sucrose, on

[159] *Z. Ver. deut. Zucker-Ind.*, 69, 403 (1919).

the contrary, has a smaller reducing effect on Ost's than on Soxhlet's solution. For these reasons Ost's solution is well adapted for the determination of invert sugar in mixtures with sucrose, and Beyersdorfer devised for this purpose a modification of Ost's method, in which the reduced copper is determined with permanganate. But the procedure has found little favor among sugar chemists. A complete description is therefore omitted.

Use of Ost's Solution for the Determination of Fructose. Biourge[160] was the first to observe that at 50° C. fructose reduces Ost's solution about ten times as strongly as glucose. Nijns,[161] who repeated Biourge's work, claimed that at 48.5–49° C. neither glucose nor sucrose has any measurable effect on Ost's solution, and that by working at that temperature fructose can be determined in the presence of the other two sugars without applying a correction. Nijns used an Ost's solution containing 15 g. of copper sulfate per liter, and a heating period of 2½ hours. Later investigations by Jackson,[162] by Schuette and Terrill,[163] and by Zerban and Sattler[164] proved, however, that glucose has a decided reducing effect, and that even that of sucrose, while small, is not negligible.

Determination of Fructose by the Method of Jackson and Mathews.[165] These authors made a further study of Nijns's method and developed a more practical procedure by increasing the copper content of Ost's solution, raising the temperature at which the reduction is carried out, and shortening the time of heating. The copper reagent is prepared by dissolving 250 g. of anhydrous potassium carbonate in about 700 ml. of hot water, and adding 100 g. of powdered potassium bicarbonate with constant stirring until all is dissolved. The mixture is cooled, and then a solution of 25.3 g. pure crystallized copper sulfate in 100 to 150 ml. of water is added with very vigorous agitation. The volume is made up to 1 liter, and the solution filtered. For the fructose determination 50 ml. of the copper reagent is placed in a 150-ml. Erlenmeyer flask, and 20 ml. of the fructose solution containing not more than 92 mg. of the sugar is added. The flask is placed in a water bath held at 55° C. within 0.1°, and left for exactly 75 minutes, the flask being agitated every 10 to 15 minutes. At the termination of the heating period the cuprous oxide is at once filtered through an asbestos mat, and the copper determined by any of the

[160] *Bull. assoc. étud. école supér. brasserie univ. Louvain*, January, 1898.
[161] *Sucr. belge*, **44**, 210 (1924).
[162] *J. Assoc. Official Agr. Chem.*, **12**, 166 (1929).
[163] *J. Assoc. Official Agr. Chem.*, **13**, 93 (1930).
[164] *Ind. Eng. Chem., Anal. Ed.*, **2**, 307 (1930).
[165] *Bur. Standards J. Research*, **8**, 403 (1932).

methods previously described. Jackson and Mathews prefer the potassium dichromate and ferrous ammonium sulfate procedure described on p. 783. The table calculated by the authors, showing milligrams of copper equivalent to milligrams of fructose, is found in the Appendix (Table 28).

Schuette and Terrill found that Ost's solutions containing more than 15.7 g. copper sulfate are not stable, but give a precipitate upon standing in the dark. They recommend therefore that two separate solutions be prepared, as in the case of Soxhlet's solution. Solution I contains 25.3 g. $CuSO_4 \cdot 5H_2O$ dissolved to 200 ml.; solution II contains 312.5 g. K_2CO_3 and 125 g. $KHCO_3$ dissolved to 1 liter as described above. Just before use 1 volume of solution I is mixed with 4 volumes of solution II.

The reducing effect of glucose varies somewhat under the above conditions with the relative quantities of glucose and fructose present. For constant amounts of glucose its reducing effect is practically independent of the fructose concentration. When the ratio of glucose to fructose is very high, however, the reducing effect of the glucose is slightly lowered. But the total variations are so small that the average value of 12.4 mg. of glucose as the equivalent of 1 mg. fructose may be used in practice without appreciable error.

The reducing effect of sucrose in the presence of glucose and fructose is very slight. It may be expressed by the following formula:

$$\text{mg. Cu} = 3.32\,S - 0.31\,S^2 + 0.27$$

where S is grams sucrose in the 20 ml. of solution.

The following table shows the milligrams of copper reduced by 1 to 5 g. sucrose, as calculated by this formula:

Sucrose, grams	1	2	3	4	5
Copper, milligrams	3.3	5.7	7.4	8.5	9.0

To correct for the effect of the sucrose, the milligrams of copper shown in this table are deducted from the milligrams of copper found before the copper is converted into its fructose equivalent.

The special field of application of Jackson and Mathews' method is in the analysis of sugar mixtures containing fructose, since this sugar, when present alone, can be determined more easily with Soxhlet's solution. The determination of fructose in mixtures with other sugars is discussed in Chapter XVI.

Kendall's Alkaline Salicylate Method. Kendall[166] has devised a method for determining reducing sugars in which salicylic acid and

[166] *J. Am. Chem. Soc.*, 34, 317 (1912).

potassium bicarbonate are used in place of the ordinary alkaline tartrate mixture of Fehling's solution. The advantages claimed are that the alkaline salicylate mixture has no copper-reducing power of its own and that much larger amounts of copper are reduced by a given weight of sugar when the carbonates of the alkalies are used in place of the hydroxides.

The sugar solution is measured into a 200-ml. Erlenmeyer flask, and the volume made up to 100 ml. with distilled water. There are then added in succession 5 g. salicylic acid, 15 ml. copper sulfate solution containing 133.33 g. $CuSO_4 \cdot 5H_2O$ per liter, and 25 ml. potassium carbonate solution containing 600 g. K_2CO_3 per liter. The flask is shaken until the salicylic acid has completely dissolved and then placed in a boiling-water bath for exactly 20 minutes; the reduced cuprous oxide is then filtered upon asbestos, washed with hot water, and the copper determined by Kendall's modified iodide method (p. 780). From the milligrams of copper thus found the corresponding weights of glucose, invert sugar, lactose hydrate, and maltose hydrate are determined from a specially calculated table.

Determination of the Copper Number of Paper.[167] In the manufacture of paper the cellulose always suffers slight decomposition. The extent of this degradation affords a measure of the probable keeping quality of the paper. It is determined by the reducing effect of the paper on alkaline copper solution, and the so-called copper number is defined as the grams of copper reduced by 100 g. of paper. Fehling's solution was first used for the determination of the copper number, but it was found impossible to duplicate the results. This difficulty has been overcome by reducing the alkalinity of the copper reagent. The paper must first be ground to a very fine pulp by means of a disintegrator which converts it into a cottonlike mass. The reagents are prepared as follows:

Solution A. Dissolve 100 g. of crystallized copper sulfate to 1 liter.
Solution B. Dissolve 50 g. of sodium bicarbonate and 350 g. of sodium carbonate, $Na_2CO_3 \cdot 10H_2O$, or the equivalent amount of anhydrous sodium carbonate, to 1 liter. The phosphomolybdic acid reagent is made by dissolving 100 g. of sodium molybdate, $Na_2MoO_4 \cdot 2H_2O$, in 75 ml. of 83 per cent phosphoric acid and 275 ml. of 95 per cent sulfuric acid, and diluting with 1750 ml. of water.

A 1.5-g. sample of the finely ground paper is transferred to a 125-ml. Erlenmeyer flask of Pyrex glass. Five milliliters of copper reagent A is added to 95 ml. of reagent B in another flask, heated to boiling, poured over the paper sample, and thoroughly mixed with it by stirring.

[167] Burton and Rasch, *Bur. Standards J. Research*, **6**, 603 (1931).

The flask is loosely stoppered with a glass bulb and placed for 3 hours in a bath heated to 100° ± 0.1° C., the contents of the flask being shaken occasionally. The mixture is then filtered on a Büchner funnel through a good grade of filter paper (Whatman No. 42), with suction. The residue on the filter is washed with 100 ml. of 5 per cent sodium carbonate solution and then with 250 ml. of water. The fiber and filter paper are transferred to a 400-ml. beaker, 25 ml. of the phosphomolybdic acid reagent is added, and the mixture is well macerated with a flattened glass rod. After a few minutes the mixture is diluted with 100 ml. of cold water, and again filtered through a Büchner funnel with suction. The filter is washed with small successive portions of water until the blue color entirely disappears. The filtrate is then titrated with 0.05 *N* potassium permanganate to a faint pink. A blank is run without the paper sample, and the copper number is calculated by the formula

$$\text{Copper number} = \frac{6.357 \times (\text{ml. } KMnO_4 \text{ for sample} - KMnO_4 \text{ for blank}) \times 0.05}{W}$$

where *W* is the weight of the sample. The latter is usually corrected for the sizing material, filler, and moisture of the paper, to reduce the figure to the cellulosic material in the paper.

Methods Employing an Excess of Copper Reagent, and Determination of the Reduced or the Unreduced Copper Without Previous Filtration

Maquenne[168] was the first to recognize that it is not necessary to filter off the cuprous oxide at all, but that the unreduced copper can be determined in the presence of the cuprous oxide precipitate by titration with potassium iodide and thiosulfate. But his method gave unsatisfactory results because he acidified first and added the potassium iodide afterward, instead of vice versa.

Shaffer and Hartmann[169] made a careful study of iodometric copper determination in alkaline copper reagents partially reduced by glucose. The reaction shown on p. 779 is reversible and may be expressed in its simplest form by the following two formulas:

$$2Cu^{++} + 4I^- \rightarrow 2CuI + I_2$$

and

$$2Cu^{++} + 2I^- \leftarrow 2Cu^{+} + I_2$$

[168] *Bull. soc. chim.*, [3], 19, 926 (1889).
[169] *J. Biol. Chem.*, 45, 349 (1920).

By choosing the proper conditions the reaction may be directed either way, and the reduced copper or the unreduced copper may be accurately determined in the presence of the other, that is without previous filtration.

To measure the unreduced copper, enough potassium iodide must be added to give a final concentration of about 0.25 *M* (4 to 5 g. in 100 ml.). To determine the reduced copper, on the other hand, the solution must be so dilute that the final concentration of copper and of iodine does not exceed about 0.005 *M*. In the latter case the reaction may be further stabilized by the addition of potassium oxalate. Because of the large quantity of potassium iodide required for the determination of the unreduced copper, this procedure is rather expensive and the end point is also not so easily seen because of the cuprous iodide suspended in the solution. But the determination of either reduced or unreduced copper requires much less time than in any of the filtration methods and is therefore preferable to them. The procedure also avoids the reoxidation of the cuprous oxide which may occur during the filtration of the solution.

A large number of methods have been based on the principle described, but only some of the more important ones are given here as examples.

A. DETERMINATION OF UNREDUCED COPPER WITHOUT FILTRATION

Shaffer and Hartmann's Modification of the Munson and Walker Method.[170] In this procedure the directions of Munson and Walker are followed up to the end of the boiling period. The solution containing the precipitated cuprous oxide is not filtered, however, but cooled quickly in running water. Six grams of potassium iodide is dissolved in it, and it is then acidified with 25 ml. of 5 *N* sulfuric acid. The liberated iodine is titrated with 0.1 *N* thiosulfate, starch being used as indicator toward the end of the titration. The total copper in the 50 ml. of Soxhlet's solution is also determined by a second titration, and the difference between the two titration results is equivalent to the cuprous oxide precipitated by the reducing sugar. Each milliliter of the difference indicates 6.36 mg. copper. The corresponding amount of reducing sugar is found from Munson and Walker's table. Shaffer and Hartmann obtained results closely agreeing with the gravimetric data of Munson and Walker, in the range from 20 to 200 mg. reducing sugar.

Schoorl's Iodide Method with Soxhlet's Solution.[171] This method is conducted as follows:

[170] *J. Biol. Chem.*, **45**, 349 (1921).

[171] *Chem. Weekblad*, **9**, 678 (1912); **12**, 481 (1915).

In a 200- to 300-ml. Erlenmeyer flask are placed 10 ml. each of Soxhlet's copper and alkaline tartrate solution, and 30 ml. of sugar solution which should not contain more than 90 mg. of reducing sugar. The flask is placed on a wire gauze covered with a piece of sheet asbestos having a circular hole of 6-cm. diameter. The solution is brought to a boil in as nearly 3 minutes as possible and kept at gentle ebullition for exactly 2 minutes. The flask is then quickly cooled to room temperature in running water, 3 g. of potassium iodide in 10 ml. of water and 10 ml. of 25 per cent sulfuric acid (1 volume concentrated acid and 6 volumes water) are then added; the solution is mixed and titrated immediately with 0.1 *N* thiosulfate solution until the liquid becomes a pale brownish yellow. Starch solution is then added and the titration carefully continued, without violent agitation, until the blue changes to a cream color. Two blank tests are also conducted upon the copper tartrate solution and 30 ml. of water. The difference between the average milliliters of thiosulfate required for the blank experiment and for the actual test gives the milliliters of 0.1 *N* thiosulfate equivalent to the reduced copper and sugar. The milligrams of glucose, fructose, invert sugar, arabinose, xylose, galactose, mannose, or rhamnose corresponding to the milliliters of thiosulfate solution are shown in Table 29 in the Appendix.

Ruoss[172] criticized Schoorl's method on the ground that the oxidation products formed by the effect of Fehling's solution on reducing sugars have the power of binding iodine, and that this causes high results. Schoorl and Regenbogen[173] showed, however, that with pure glucose the error due to this cause is less than 0.07 ml. of 0.1 *N* thiosulfate solution and can be neglected. Furthermore, filtration of the precipitated cuprous oxide and determination of the copper in the precipitate by the permanganate method gave the same results as the iodine titration method.

However, Schoorl's method is adapted only to quantities of reducing sugars below 90 mg. This is a disadvantage in comparison with other methods when large amounts of reducing sugars are present, because of the greater dilution and consequently greater multiplication of errors.

Schoorl's Method, Modified by the Java Sugar Experiment Station, for Determining Invert Sugar. Schoorl[174] overcame the above objection by operating with 50 ml. instead of 20 ml. of Soxhlet's solution, and increasing the quantity of potassium iodide and sulfuric acid.

[172] *Z. anal. Chem.*, **55**, 1 (1916).
[173] *Z. Ver. deut. Zucker-Ind.*, **67**, 563 (1917).
[174] *Arch. Suikerind.*, **24**, 1967 (1916).

Van de Kreke[175] made a further study of this modification and on the basis of the results obtained the Java Sugar Experiment Station adopted the following method[176] for estimating invert sugar in juices and molasses. Soxhlet's solution is used, and the Herzfeld procedure of reduction (p. 806) is followed. In order to prevent overheating, a few pieces of pumice stone which has previously been boiled out and ignited, or a little powdered talc, is added. At the end of the boiling period no cold water is added, but the flask is quickly cooled in running water. Then 25 ml. of a 20 per cent solution of potassium iodide and 35 ml. of dilute sulfuric acid (1 volume of concentrated acid plus 5 volumes of water) are added, and the liberated iodine is titrated with 0.1 *N* sodium thiosulfate solution. Toward the end of the titration a few milliliters of 1 per cent starch solution are added. At the end point the color changes from purple to cream yellow. The milliliters of thiosulfate used are deducted from the milliliters found in a blank test run with 50 ml. of water instead of sugar solution, and the difference, multiplied by 6.357, gives milligrams of copper reduced. The corresponding milligrams of invert sugar are found by means of Meissl and Hiller's factors (p. 810). The Java Sugar Experiment Station has also calculated special tables for invert-sugar determinations in cane juices and molasses.

Although this method permits the determination of larger quantities of invert sugar than Schoorl's original procedure, the expense for iodide is greater. As Pick, Vondrák, and others have shown (see p. 808), the addition of talc powder and similar materials affects the amount of copper reduced, and the use of the original Meissl and Hiller factors may thus give erroneous results. The factors can be used without change only if the mixture of Soxhlet's and sugar solution is boiled without any additions, as prescribed by Herzfeld.

Schoorl's Iodide Method with Luff's Solution for Determining Invert Sugar in Cane Sugars.[177] In order to minimize the reducing effect of sucrose in the determination of invert sugar, Schoorl introduced the use of Luff's copper carbonate solution[178] instead of Soxhlet's reagent. Luff's solution is similar to Ost's, but contains citric instead of tartaric acid. Like Ost's reagent, it keeps much better than the mixed Soxhlet reagent and shows no autoreduction. Small variations in the boiling period affect the result much less than in the case of Soxhlet's

[175] *Arch. Suikerind.*, **34**, III, 411 (1926).

[176] *Proefstat. Java-Suikerind., Bull.* **11**, 3d ed., 1921; 4th ed., 1927; 5th ed., 1931.

[177] *Chem. Weekblad*, **22**, 132 (1925).

[178] *Z. ges. Brauw.*, **21**, 392, 410 (1898).

solution, and the precision is therefore greater. The reducing effect of sucrose is very slight. The method was adapted by van de Kreke[179] for the determination of small quantities of invert sugar in the presence of large amounts of sucrose, and the Java Sugar Experiment Station employs the procedure, in the place of Herzfeld's, for estimating invert sugar in cane sugars.

Luff's solution is prepared by dissolving 17.3 g. crystallized copper sulfate and 115 g. citric acid in 200 ml. water in a large flask (about 2-liter capacity). Then a solution of 185.3 g. anhydrous sodium carbonate in 500 ml. water is slowly added with constant agitation. If

TABLE CXIV[180]

DETERMINATION OF INVERT SUGAR IN SUGARS ACCORDING TO LUFF-SCHOORL

0.1 *N* Thiosulfate ml.	No Sucrose	1.25 g. Sucrose	2.5 g. Sucrose	5.0 g. Sucrose
	mg. Invert Sugar in 25 ml. solution			
1	3.20	2.75	2.50	1.90
2	6.20	5.80	5.55	5.00
3	9.15	8.90	8.55	8.05
4	12.10	12.00	11.50	11.05
5	15.10	15.00	14.50	14.05
6	18.10	18.00	17.50	17.05
7	21.00	21.00	20.50	20.10
8	24.00	24.00	23.50	23.10
9	27.00	27.00	26.60	26.15
10	30.10	30.20	29.80	29.20
11	33.20	33.40	33.00	32.30
12	36.30	36.60	36.20	35.45
13	39.50	39.85	39.40	38.60
14	42.80	43.10	42.60	41.70
15	46.05	46.30	45.80	44.90
16	49.35	49.65	49.60	48.40

the sodium carbonate is not entirely free from water it must be analyzed and a quantity equivalent to 185.3 g. of anhydrous salt weighed out. When the evolution of carbon dioxide has ceased the solution is transferred to a 1-liter volumetric flask and made up to the mark. Then 2 g. of ignited and washed Filter-Cel is added and the solution filtered through a Büchner funnel. Twenty-five milliliters each of Luff's solution and of the clarified sugar solution are transferred to a 300-ml. Erlenmeyer flask which is provided with a vertical reflux condenser. The flask is placed on a wire gauze covered with a piece of asbestos sheet having a hole of about 6-cm. diameter. The liquid is heated to

[179] *Arch. Suikerind.*, 37, III, 781 (1929).

[180] Douwes Dekker, *Arch. Suikerind.*, 42, I, 629 (1934). Here condensed. The original table is in steps of 0.1 ml. thiosulfate.

boiling in about 3 minutes, and the boiling continued gently for exactly 5 minutes longer. The flask is cooled immediately in running water. Fifteen milliliters of a 20 per cent solution of potassium iodide is added, and then 25 ml. of dilute sulfuric acid (1 volume concentrated acid plus 5 volumes of water) is run in slowly, with careful rotation of the flask. When the evolution of carbon dioxide ceases the liberated iodine is titrated with 0.1 *N* thiosulfate solution, as described in the preceding method. A blank titration is run with 25 ml. of water instead of sugar solution, and the titer of the sugar solution deducted from that of the blank. The milligrams of invert sugar corresponding to milliliters thiosulfate are found from Table CXIV.

Modified Luff-Schoorl Method. Schoorl[181] later found that, when a copper citrate solution with more copper and less citric acid than that previously specified by him is employed, and the boiling period extended to 10 minutes, glucose and fructose show exactly the same reducing power. Although this modification introduces no new principle, the method is described here, because it is used in Kruisheer's method for analyzing complex sugar mixtures (see Chapter XVI).

The copper reagent is prepared as follows: 388 g. of crystallized sodium carbonate ($Na_2CO_3 \cdot 10H_2O$) is dissolved in 300 to 400 ml. of lukewarm water. A solution of 50 g. citric acid in 50 ml. of water is added, and then a solution of 25 g. crystallized copper sulfate, free from iron, in about 100 ml. of water. The mixture is allowed to cool and made up to 1 liter. After a few days' standing, the clear solution is decanted or siphoned off. It keeps indefinitely and shows no autoreduction even upon boiling.

Place 25 ml. of this reagent, and 25 ml. of sugar solution in a 300-ml. Erlenmeyer flask, add a few pieces of pumice stone, and heat over a free flame, holding the flask by the hand, so that the solution begins to boil in about 2 minutes. Place the flask on a wire gauze covered with an asbestos screen, as in Herzfeld's method, connect with a reflux condenser, and boil for exactly 10 minutes longer. Cool at once in running water, and after 5 minutes add 3 g. of potassium iodide. Acidify with 20 ml. of 25 per cent hydrochloric acid, and shake until the evolution of gas stops. The remaining foam may be removed by a few drops of ether. Titrate the liberated iodine with 0.1 *N* thiosulfate, using 1 ml. of 2 per cent starch solution toward the end, until the blue color disappears and the precipitate is cream colored. Run a blank with 25 ml. of the copper reagent and 25 ml. of water. The difference between the two titrations is equivalent to the reduced copper and to the quantity of reducing sugar present, which is found from Table CXV.

[181] *Chem. Weekblad*, **26**, 130 (1929).

TABLE CXV

0.1 *N* Thiosulfate	Glucose, Fructose, Invert Sugar	Anhydrous Lactose	Anhydrous Maltose
ml.	mg.	mg.	mg.
1	2.4	3.6	3.9
2	4.8	7.3	7.8
3	7.2	11.0	11.7
4	9.7	14.7	15.6
5	12.2	18.4	19.6
6	14.7	22.1	23.5
7	17.2	25.8	27.5
8	19.8	29.5	31.5
9	22.4	33.2	35.5
10	25.0	37.0	39.5
11	27.6	40.8	43.5
12	30.3	44.6	47.5
13	33.0	48.4	51.6
14	35.7	52.2	55.7
15	38.5	56.0	59.8
16	41.3	59.9	63.9
17	44.2	63.8	68.0
18	47.1	67.7	72.2
19	50.0	71.7	75.5
20	53.0	75.7	80.9
21	56.0	79.8	85 4
22	59.1	83.9	90.0
23	62.2	88.0	94.6

The same table may also be employed if larger or smaller quantities of the copper reagent than the 25 ml. specified are used, as for example 50 ml. reagent plus 50 ml. sugar solution. The titer found is divided by 2, the corresponding milligrams of sugar found from the table, and the result multiplied by 2. Thus, a titer of 20 under these conditions indicates 25.0 $\times$ 2, or 50 mg. glucose, and not 53.0 mg. On the other hand, if 10 ml. copper reagent and 10 ml. of sugar solution are used, and the titer found is 6 ml., this figure is multiplied by 2.5, giving 15, and the corresponding milligrams of glucose, 38.5, are divided by 2.5, giving 15.4 mg., instead of 14.7 mg.

Bruhns's Iodide-Thiocyanate Method for Determining Invert Sugar.[182] Bruhns made the important observation that in the estimation of unreduced copper the potassium iodide can be replaced, for the larger part, by the much cheaper thiocyanate. The cuprous iodide formed in the reaction

$$2CuSO_4 + 4KI \longrightarrow 2K_2SO_4 + 2CuI + I_2$$

is converted by the thiocyanate into cuprous thiocyanate:

$$2CuI + 2KCNS \longrightarrow 2CuCNS + 2KI$$

[182] *Centr. Zuckerind.*, 25, 732 (1917); *Deut. Zuckerind.*, 54, 1237, 1316, 1337 (1929); 55, 120, 422, 486 (1930); 58, 287 (1933).

When the solution is titrated with thiosulfate, additional iodide is formed from the iodine liberated according to the first equation, more cupric ions react with the iodide, and these transformations go on until all the copper has been converted into cuprous thiocyanate with the formation of an equivalent amount of iodine which is titrated with thiosulfate. While the first reaction is reversible, and cuprous iodide is oxidized by iodine except when a large excess of potassium iodide is present, the cuprous thiocyanate is not attacked at all by iodine, and an excess of potassium iodide is unnecessary.

The following reagents are used: Fehling's solution I consists of approximately 70 g. pure crystallized copper sulfate per liter. Since the copper content of the mixed solution is always determined by a blank titration, the sulfate need not be weighed out exactly. The alkaline tartrate solution II is the same as Soxhlet's, containing 346 g. Rochelle salt and 100 g. sodium hydroxide per liter. The Rochelle salt must be of the highest purity; the sodium hydroxide must be free from nitrites and contain not more than traces of iron or carbonate. Ten milliliters of the alkaline tartrate solution should neutralize 23.6 to 24 ml. of normal acid, using phenolphthalein as indicator. Upon standing this solution usually deposits a flocculent precipitate, but this does not interfere and need not be removed by filtration; the clear liquid can be pipetted off from the top.

The iodide thiocyanate reagent is prepared by dissolving 10 g. potassium iodide, which must be free from iodate, nitrite, and iron, and 65 g. chemically pure potassium thiocyanate in water, and completing the volume to 250 ml. with the addition of 1 ml. of *N* sodium hydroxide. The solution is kept in a brown, well-stoppered bottle and must always remain alkaline toward phenolphthalein.

The thiosulfate solution is made 0.1387 *N*, so that 1 ml. equals 1 ml. Fehling's solution, by dissolving 34.5 g. of sodium thiosulfate to 1 liter with the addition of 2 ml. *N* sodium hydroxide. The solution is titrated against a standard solution containing 6.802 g. potassium dichromate per liter, and the volume of the thiosulfate solution is adjusted so that 20 ml. of it is equivalent to 20 ml. of the dichromate solution.

The sulfuric acid used to acidify the solution before titrating with thiosulfate is prepared by pouring 150 ml. of concentrated acid, which must contain no nitrous acid, into 850 ml. of water. The starch indicator is made by adding a suspension of 1 g. soluble starch in a few milliliters of cold water to 90 ml. of boiling water. It should be prepared fresh at frequent intervals.

Transfer 10 ml. each of copper solution and alkaline tartrate solution,

and 20 ml. of sugar solution, to a 200-ml. Erlenmeyer flask, place it on a wire gauze covered with a piece of asbestos board having a hole 6 cm. in diameter, heat to boiling, and boil for exactly 2 minutes. During the first heating period sprinkle some finely powdered talc on the surface of the liquid, but do not agitate the flask. At the end of the 2 minutes' boiling remove the flask at once from the flame, add 50 ml. of cold water saturated with air, place a small beaker over the mouth of the flask, and cool in running water to 15° C. or lower. This point is important, since at higher temperatures erroneous results are obtained in the subsequent titration. Add 2.5 ml. of the iodide thiocyanate solution and 10 ml. of the dilute sulfuric acid, and mix. Titrate immediately with the thiosulfate solution until the brown color obtained at the beginning changes to gray. Then add 3 ml. of starch indicator and continue the titration until the precipitate becomes leather-yellow to red, depending on the quantity of copper reduced, and the color does not revert to blue or gray within 5 minutes. A blank titration is run with 20 ml. of water instead of sugar solution, and the titer found with the sample is deducted from the result of the blank. If the sugar solution contains any substances which are oxidized by iodine, the blank is run with 20 ml. of the sugar solution, omitting the boiling and operating entirely in the cold. But in this case one must assure himself that a blank run with 20 ml. of water in the cold gives the same result as 20 ml. of water after boiling under the prescribed condition.

Bruhns has applied this method to the determination of glucose, fructose, invert sugar, lactose, and invert sugar in the presence of varying amounts of sucrose. The 20 ml. of sugar solution must not contain over 75 mg. of invert sugar or 116 mg. of lactose. Bruhns's tables are reproduced in the Appendix, Table 30.

The titration with thiosulfate must be carried out immediately and very rapidly after the iodide thiocyanate and sulfuric acid have been added. If the solution is allowed to stand for any length of time before titration the cuprous ions react with thiocyanogen ions with the formation of free thiocyanogen, and this decomposes further, giving rise to hydrogen cyanide, ammonia, carbon dioxide, and urea, as has been shown by Krüger and Tschirch.[183] Much practical experience is necessary to judge the exact end point in the titration, because the color of the precipitate varies widely, expecially when low-purity products are analyzed. But the rapidity of the method and the lower cost of the reagents, compared with the straight iodide method, have gained it many adherents.

Schoorl applied Bruhns's thiocyanate modification to his iodometric

[183] *Z. anal. Chem.*, **97**, 161 (1934).

methods using Soxhlet's (p. 828) and also Luff's (p. 830) solutions,[184] but subsequent investigations at the Java Sugar Experiment Station by van de Kreke[185] showed that the end point is not so good as when an excess of potassium iodide alone is used, and these modified methods have not come into extended use.

B. DETERMINATION OF REDUCED COPPER, WITHOUT FILTRATION

In methods of this type the precipitated cuprous oxide is brought into solution in the presence of the excess copper reagent, and the cuprous ion is determined with standard iodine solution. The finding of Shaffer and Hartmann, that the final concentration of copper and of iodine must not exceed 0.005 *M*, has already been referred to. If the concentrations of the reagents are properly chosen, as much as 200 mg. reducing sugar in 50 ml. solution may be determined by methods based on this principle, but they are used more especially for the estimation of small quantities of reducing sugars, such as occur in biological fluids like urine and blood, or in beet and high-purity cane products. The high precision of the iodometric method renders it particularly suitable for these purposes. Among the many methods in this category, those of Shaffer and Hartmann, Ofner, Spengler, Kraisy, Scales, Bang, and Shaffer and Somogyi will be described here.

Shaffer and Hartmann's Modification of the Munson and Walker Method. [186] The iodine for the oxidation of the cuprous oxide is added in the form of an iodate-iodide solution, which is prepared by dissolving 5.4 g. potassium iodate and 60 g. potassium iodide in water, adding a small amount of sodium hydroxide solution to prevent oxidation, and diluting to 1 liter. The Munson and Walker procedure (p. 800) is followed up to the end of the boiling period. The reaction mixture is at once cooled in running water to about 30° to 35° C., and 50 ml. of the iodate-iodide solution (for small amounts of cuprous oxide 25 ml. is sufficient) is added. The mixture is acidified rapidly with 15 to 17 ml. of 5 *N* sulfuric acid, from a graduate or a pipette with wide opening, and the liquid agitated until all the cuprous oxide has either been dissolved or converted into iodide. Then 20 ml. of a saturated solution of potassium oxalate is added. When the solution has become clear, the excess iodine is titrated back with 0.1 *N* solution of thiosulfate, starch

[184] Schoorl and Kolthoff, *Pharm. Weekblad,* **55**, 344 (1918); Schoorl, *Chem. Weekblad,* **22**, 285 (1925); **26**, 130 (1929).

[185] *Arch. Suikerind.*, **37**, III, 781 (1929).

[186] *J. Biol. Chem.*, **45**, 349 (1920). The oxidation of the cuprous oxide proceeds according to the equation:

$$KIO_3 + 5KI + 9H_2SO_4 + 3Cu_2O \rightarrow 6CuSO_4 + 3K_2SO_4 + 6HI + 6H_2O.$$

being used as indicator toward the end of the titration. A blank titration is run with 50 ml. of Soxhlet's solution and 50 ml. of water. The milliliters of thiosulfate used in the blank less the milliliters of thiosulfate used in the sugar titration, multiplied by 6.36, gives milligrams of copper reduced. The corresponding milligrams of reducing sugar are found from Munson and Walker's table (Appendix, Table 19A). Shaffer and Hartmann's results by this method checked closely with those of the gravimetric procedure, within the limits of 20 and 200 mg. reducing sugar. An increasing number of biochemists are using this method, with satisfactory results.

Ofner's Iodometric Method for Determining Invert Sugar in Beet Products. Ofner[187] found that a copper carbonate solution, prepared with a large excess of Rochelle salt and containing sodium phosphate in addition, is under certain experimental conditions not reduced to any extent by sucrose, and that such a solution is therefore especially suitable for determining small quantities of invert sugar in mixture with large amounts of sucrose. In the original method the copper precipitate was filtered off and weighed as metallic copper. The procedure was then changed to differential titration according to Bruhns[188] (p. 833). In a further modification titration of the precipitated cuprous oxide is employed, and this method is carried out as follows.[189]

The copper reagent is prepared by dissolving in a 1-liter volumetric flask 5.0 g. crystallized copper sulfate, 10 g. anhydrous sodium carbonate, 300 g. powdered Rochelle salt, and 50 g. crystallized secondary sodium phosphate ($Na_2HPO_4 \cdot 12H_2O$) in water to a total volume of about 900 ml., by shaking at room temperature and then placing the flask for 2 hours on a boiling-water bath. After cooling, the volume is completed to the mark; the solution is filtered with the aid of decolorizing carbon and kept in a well-stoppered bottle of dark glass. If mold should develop in the solution upon prolonged standing it should be refiltered with activated carbon. The reagent shows no autoreduction. The reducing effect of sucrose is very small, each gram of sucrose being equivalent to 0.1 mg. of invert sugar. The standard iodine solution contains 2.050 g. chemically pure iodine and 10 g. potassium iodide, free from iodate, dissolved in a small quantity of water, and made up to 500 ml. Its strength is such that 1 ml. of it corresponds to 1 mg. of invert sugar under the experimental conditions employed. The iodine solution is also kept in a dark bottle with well-fitting glass stopper.

[187] *Z. Zuckerind. čechoslovak. Rep.*, **49**, 279 (1924/25); **50**, 65 (1925/26).
[188] *Z. Zuckerind. čechoslovak. Rep.*, **53**, 733 (1928/1929).
[189] *Z. Zuckerind. čechoslovak. Rep.*, **56**, 249 (1931/32); **58**, 33 (1933/34); Ofner and Gračko, *ibid.*, **57**, 125 (1932/33).

The thiosulfate solution is made by dissolving 4 g. sodium thiosulfate in water, adding 2 ml. of 0.1 *N* sodium hydroxide to prevent decomposition, and diluting to 500 ml. total volume; the solution is accurately standardized with the iodine solution.

For the analysis of beet raw sugars containing not over 0.15 per cent invert sugar, 153.7 ml. of the filtrate used for the direct polarization,[190] and containing 40 g. of the sugar, is transferred to a 200-ml. volumetric flask and deleaded with 15 ml. of a solution of crystallized secondary sodium phosphate (100 g. to 1 liter), and the volume is completed to the mark. Then 1 g. of activated carbon is added, the flask is well shaken, and allowed to stand for 15 minutes, and the solution is filtered. Ofner claims that the carbon removes reducing non-sugars without affecting the invert sugar content. Fifty milliliters of the filtrate is used for the determination. White sugars require no preliminary treatment, and a solution containing 10 g. of sugar in each 50 ml. is used directly for the analysis.

The 50 ml. of sugar solution is transferred to a 300-ml. Erlenmeyer flask and mixed with 50 ml. of the copper reagent, and a small quantity of powdered pumice stone or talcum is added to prevent overheating during the boiling period. The flask is placed on a wire gauze resting on an asbestos plate with a round hole about 6 cm. in diameter in the center. The liquid is heated to boiling in 4 to 5 minutes and then gently boiled with a smaller flame for exactly 5 minutes. The flask is then rapidly cooled in running water, and 15 ml. of *N* hydrochloric acid (82 ml. concentrated acid diluted to 1 liter) is carefully run in along the inner wall of the tilted flask. Immediately after acidifying the standard iodine solution is added, the flask being slowly revolved after the first few milliliters have been run in. The iodine must be added in excess; a total of 5 to 20 ml. is used, depending on the amount of invert sugar present. The flask is immediately stoppered after the addition of the iodine, which is allowed to act for 2 minutes, during occasional agitation. Then 5 ml. of a 0.5 per cent starch solution is added, and the excess iodine is titrated back with the standard thiosulfate. The difference between the milliliters of iodine solution added from the burette and the milliliters of thiosulfate used in the back titration directly gives milligrams of invert sugar in 10 g. of sugar. A correction must be applied for the reducing effect of the sucrose, amounting to 1 ml. iodine solution = 1 mg. invert sugar.

This method has been found to be well suited for routine work, and

[190] The lead subacetate used for clarifying prior to polarization is supposed not to affect the invert-sugar content of beet sugars. For a discussion of this subject see pp. 882–885.

it has been adopted officially in Czechoslovakia for the determination of invert sugar in beet sugar. If the invert-sugar content exceeds 0.15 per cent but not 0.3 per cent, 5 g. of the sugar is taken for the analysis and a correction of 0.5 mg. is applied to the milligrams of invert sugar found, for the reducing effect of the sucrose. In the case of white sirups, a quantity containing 10 or 5 g. sucrose respectively, is diluted to 50 ml. for the analysis.

Low-grade beet sugars are clarified with larger quantities of lead subacetate and sodium phosphate than first sugars, and with 1 g. carbon for the double-normal weight. Otherwise the analysis is carried out in the same manner as described above.

With beet molasses and low-purity sirups the boiling period is increased to 7 minutes. The solutions are clarified with neutral lead acetate solution, deleaded with sodium phosphate, and treated with a minimum quantity of activated carbon, sufficient to remove reducing non-sugars without affecting the invert-sugar content. The concentration is chosen so that 50 ml. of the final filtrate contains 5 g. of molasses. Hence each milliliter of iodine solution required to oxidize the cuprous oxide indicates 0.02 per cent invert sugar. If the molasses contains more than 0.3 per cent, an aliquot of the final filtrate is diluted to 50 ml. before the addition of the Fehling's solution. A correction of 0.1 ml. iodine is applied for each gram of sucrose in the 50 ml. of molasses solution. Another correction must be made for the reducing non-sugars not removed by the clarification process. For this purpose another test is made exactly as the analysis itself, but in the cold, and the milliliters of iodine found are deducted from the milliliters found in the actual analysis.

In a simplified modification of the method, suitable for routine tests in factory control, clarification is omitted entirely, but the reducing non-sugars are removed by adding 5 ml. of the iodine solution to the 50 ml. of sugar solution and 50 ml. of Fehling's solution before the mixture is boiled. The 5 ml. of iodine solution thus added is not considered in calculating the result of the later titration. When a solution containing less than 10 g. of sucrose in 50 ml. is used for the analysis, the quantity of iodine added for removal of the non-sugars is correspondingly reduced.

Fischl[191] claims that Ofner's solution can be used for the estimation of fructose in the presence of glucose. A mixture of 10 ml. of sugar solution containing not over 0.5 per cent sugars, and 30 ml. of Ofner's reagent, is heated in a water bath at 60° C. for 5 minutes, and the reduced copper is determined iodometrically with $N/77$ iodine solution.

[191] *Chimie & industrie*, Special Number, June, 1933, p. 1123.

Each milliliter of this is equivalent to 5 mg. of fructose. This method, which is similar to that of Jackson and Mathews (p. 824), requires further study.

Method of Spengler, Tödt, and Scheuer for Determining Invert Sugar in Beet Sugars.[192] This method is similar to that of Ofner, but the reduction is carried out in a boiling-water bath, and Müller's solution is used as the copper reagent. Under these conditions 1 equivalent of invert sugar reduces exactly 6 equivalents of copper (see p. 822), and slight differences in the heating period have no appreciable effect on the result. Müller's solution is prepared as follows: Dissolve 35 g. of crystallized copper sulfate in 400 ml. boiling water in a 1-liter volumetric flask. In another vessel dissolve 173 g. of sodium potassium tartrate and 68 g. anhydrous sodium carbonate (or 180 g. of $Na_2CO_3 \cdot 10H_2O$) in 500 ml. of boiling water. After cooling, transfer and wash the sodium carbonate solution into the copper sulfate solution, and make up to the mark with distilled water. Only the highest-grade chemicals should be used. Add 2 teaspoons of activated carbon, mix well, let stand for several hours, and filter through a hardened filter under suction. The solution keeps for long periods of time without change. If a precipitate forms on standing the solution must be refiltered.

Ten grams of the beet sugar is dissolved in distilled water in a 300-ml. Erlenmeyer flask, and the volume is completed to 100 ml. Ten milliliters of Müller's solution is added, and the flask is placed for 10 minutes in a water bath which must be heated so that the boiling does not stop when the flasks are immersed in it. The level of the liquid in the flasks should be at least 2 cm. below the water level of the bath. At the end of the 10 minutes the flask is rapidly cooled, without agitation, under the water tap, a small beaker being placed over the mouth. The solution is then acidified with 5 ml. of 5 *N* acetic or tartaric acid, and immediately an excess of *N*/30 iodine solution (20 to 40 ml.) is added. When the precipitate has completely dissolved, the excess iodine is titrated back with *N*/30 sodium thiosulfate solution, which has been stabilized by the addition of 3 ml. of *N* sodium hydroxide per liter. A blank is run in the same manner with 100 ml. water, but without sugar. The difference between the milliliters thiosulfate used in the blank and in the test on the sample gives the milliliters *N*/30 iodine used for oxidation of the cuprous oxide. Each milliliter corresponds to 1 mg. invert sugar, or 0.01 per cent if 10 g. of sugar is used for the test.

Two corrections must be applied to the result. Two milliliters of iodine solution must be deducted for the reduction due to 10 g. of

[192] *Z. Ver. deut. Zucker-Ind.*, **86**, 130, 322 (1936).

sucrose. The effect of any reducing non-sugars present (sulfur dioxide, etc.) is corrected for by running another experiment with sugar solution and Müller's solution, but in the cold, and deducting the milliliters of iodine solution required.

The method gives satisfactory results only with sugars containing not more than 0.2 per cent invert sugar. Above that figure the results are about 10 per cent too high. But normal beet sugars do not contain over 0.2 per cent invert sugar. If the invert-sugar content is higher, less than 10 g. of sugar must be used for the test, and the correction for the reducing effect of the sucrose must be reduced proportionately, 1 ml. iodine solution for 5 g. sugar, etc.

The same method has been applied to the analysis of other beet-factory products by Spengler, Zablinsky, and Wolf.[193]

Beets and Cossettes. The normal weight (26 g.) of the finely pulped sample is digested at room temperature with 177 ml. of water, without the addition of clarifying agent, for 30 minutes, and the mixture filtered. Fifty milliliters of the filtrate is diluted to 100 ml., and the invert sugar is determined. Or 21.8 ml. of pressed beet juice, corresponding to 26 g. of beet, is diluted, without clarification, to 200 ml., and 50 ml. of this solution, diluted to 100 ml., is used for the determination.

Raw Juice. Ten milliliters of juice is diluted to 100 ml., without clarification, and the invert sugar is determined in this solution.

Thin or Thick Juice. These products usually contain lime or sulfur dioxide. If more than 5 mg. calcium oxide is present in the sample used for the determination, it must first be removed by the addition of sodium phosphate or phosphoric acid, depending on the alkalinity of the product. The solution is then diluted to 100 ml. without previous filtration. In the presence of sulfur dioxide, the sample is acidified with a few drops of dilute acetic acid, and the sulfur dioxide oxidized by adding $N/30$ iodine solution from a burette until starch indicator remains blue for a few seconds. The determination is then finished as usual, but the same quantity of iodine solution as was used for the oxidation of the sulfur dioxide must be added in the blank test.

Molasses. Twenty-two grams of sample is dissolved in water in a 200-ml. volumetric flask, clarified with neutral lead acetate solution, the solution made to the mark, and filtered. A 100–110-ml. flask is filled with the filtrate to the 100-ml. mark. The solution is deleaded with sodium carbonate solution, the volume completed to the 110-ml. mark, and the solution is filtered again. Twenty milliliters of the final filtrate, equal to 2 g. molasses, is used for the determination. If the solution is so dark that the end point cannot be recognized, the reduction

[193] *Z. Ver. deut. Zucker-Ind.*, 88, 280 (1938).

is carried out in a 500-ml. Erlenmeyer flask, and the solution diluted with 100 ml. of water just prior to the titration with thiosulfate. Any sulfur dioxide present in the molasses is first oxidized as described for thin and thick juices.

The reducing effect of the sucrose present must always be corrected for by deducting 0.2 ml. *N*/30 iodine for each gram of sucrose.

Kraisy's Method for Determining Invert Sugar in Refined Sugars.[194] Kraisy employed for this purpose the following copper reagent: solution I, containing 7.86 g. crystallized copper sulfate (= 2 g. copper) in 1 liter; solution II, containing 3.292 g. anhydrous sodium carbonate and 20 g. Rochelle salt in 1 liter. Twenty-five milliliters each of these solutions is transferred to a 250-ml. Erlenmeyer flask. In another similar flask a solution of 10 g. of the refined sugar in a total volume of 50 ml. is mixed with 5 ml. *N*/10 potassium bicarbonate solution (10.01 g. per liter). Both solutions are heated to boiling, each over a large burner, on a wire gauze covered with a piece of asbestos board having a hole about 6 cm. in diameter, the flames being so regulated that the sugar solution begins to boil when the copper solution is already boiling. It should take 2¼ to 2¾ minutes to bring the sugar solution to boiling. At this moment the boiling copper solution is poured into the sugar solution, and the boiling is continued for exactly 10 minutes, with a small flame. Then 50 ml. of freshly boiled and cooled water is added carefully, without drawing air bubbles into the solution, and without agitating the flask, which is then cooled for 5 minutes longer in cold water. The solution is next acidified with 1 to 1.25 ml. of 4 *N* hydrochloric acid (1 volume concentrated acid plus 2 volumes water), and immediately an excess of *N*/63.57 iodine solution (1 ml. = 1 mg. copper) is added. The cuprous oxide must dissolve completely, and an excess of about 5 ml. of iodine solution should be used. This is then titrated back with *N*/63.57 thiosulfate solution, using starch as indicator. The addition of thiosulfate is continued until the blue color of the iodine-starch does not return for at least 10 minutes. The difference between the milliliters of iodine added, and the milliliters of thiosulfate used in the back titration is equal to the number of milligrams of copper reduced. A blank titration is run with 50 ml. of water instead of sugar solution, and the result applied as a correction to that of the actual test. Each milligram of copper corresponds to 0.435 mg. invert sugar, or 0.00435 per cent when 10 g. of sugar is used for the analysis. This proportionality holds up to 0.05 per cent invert sugar, which is well beyond the limit for refined sugars. A final correction must be applied for the reducing effect of the sucrose

[194] *Z. Ver. deut. Zucker-Ind.*, 71, 123 (1921).

itself. Kraisy found that 10 g. of the purest sugar reduced 1.7 mg. copper, which should be deducted from the result found. But Šandera and Mirčev[195] were able to prepare sucrose which reduced only 0.66 mg. of copper, and have used this figure as a correction.

A number of refined sugars have been analyzed by this method in the New York Sugar Trade Laboratory. Among them was one medium granulated sugar which gave only 0.8 mg. copper, corrected for the water blank. This is only about one-half of that found by Kraisy, and very close to that found by Šandera for pure sucrose. This shows that the correction to be applied for the reducing power of the sucrose itself is rather uncertain. It is preferable to omit this correction altogether, especially since the invert sugar calculated without it checks very closely with that found by Main's pot method (p. 818).

Scales's Method for Determining Small Quantities of Glucose. Scales[196] employed a copper reagent which is a modification of one first proposed by Benedict,[197] and is similar to Luff's solution (p. 830). The reagents and the procedure used are described as follows by the Association of Official Agricultural Chemists, which has adopted this method:[198]

Benedict's solutions. Dissolve 16 g. of $CuSO_4 \cdot 5\,H_2O$ in 125–150 ml. of water. Then dissolve 150 g. of sodium citrate, 130 g. of sodium carbonate (anhydrous), and 10 g. of sodium bicarbonate in about 650 ml. of water, heating to accelerate solution. Combine the two solutions with stirring. Cool, make to 1 liter, and filter.

Transfer 20 ml. of the copper reagent to a 300-ml. Erlenmeyer flask fitted with a two-holed rubber stopper. Add 10 ml. of sugar solution containing less than 20 mg. of reducing sugar. Place over a flame, bring to boiling in 4 minutes, and continue the boiling for exactly 3 minutes. (Approximate conditions, flame 50 mm.; cone 20 mm.; asbestos gauze 30 mm. above burner. If preferred, an electric hot plate may be used, in which case a period of 5 minutes is required to raise the solution to the boiling point.) At the expiration of 3 minutes from the beginning of the boiling, cool rapidly by holding under a cold-water faucet, add 100 ml. of acetic acid solution (24 ml. of glacial acetic acid per liter) from a graduate, and transfer an exactly measured amount of 0.04 *N* iodine solution. Add 25 ml. of hydrochloric acid (60 ml. per liter) from a pipette held against the side of the flask, and agitate to distribute the acid rapidly. Rotate the flask for 1 minute to insure the solution of all cuprous chloride. Titrate excess iodine with 0.04 *N* thiosulfate solution, using starch solution as an indicator.

[195] *Z. Zuckerind. čechoslovak. Rep.*, **58**, 193 (1933/34).
[196] *J. Ind. Eng. Chem.*, **11**, 747 (1919).
[197] *J. Biol. Chem.*, **3**, 101 (1907).
[198] "Methods of Analysis, A. O. A. C.," 5th ed., pp. 499–500, 1940.

For amounts less than 20 mg. of sugar each milliliter of thiosulfate will represent a constant quantity of sugar; for glucose, approximately 1.12 mg. per ml. (For accurate work the analyst should determine the conversion factor for the particular conditions under which he is working by using control solutions of the pure sugars under examination.)

The method, with slight alterations, has been applied by Roy and Hughes[199] to the determination of glucose and of sucrose (after inversion) in plant juices and tissues. The results checked satisfactorily with those obtained by the Munson and Walker method.

Scales's Method, as Modified by Isbell, Pigman, and Frush.[200] These authors observed that small variations in the 3-minute boiling period used by Scales cause erratic results. The boiling time was therefore increased to 6 minutes, and the flask immersed immediately afterward in an ice-water bath for 45 seconds, being gently agitated with a circular motion. Even with these precautions the results vary slightly with the details of manipulation, and it is best for each chemist to establish his own factors.

With this modified method, the factors by which the milliliters thiosulfate must be multiplied to calculate the milligrams sugar were found to increase with the concentration of each sugar. These factors have been determined by Isbell, Pigman, and Frush for a large number of common and rare sugars, and the results are shown in Table CXVI.

The analytical results on which the factors are based varied not more than 1 per cent for the same operator, but as much as 2 per cent for different operators. The significance of the figures given in the last column of the table is discussed on p. 795.

Bang's Iodometric Method for Determining Glucose. In Bang's original method[201] a copper reagent containing carbonate and bicarbonate was used and the amount of unreduced copper determined by titrating with hydroxylamine sulfate solution in the presence of thiocyanate to the disappearance of the blue color of the solution. Other investigators obtained irregular results with this method and found it difficult to judge the end point in the presence of colored impurities. For this reason Bang[202] changed both the composition of the reagent and the procedure of copper determination. The modified copper solution is prepared by dissolving 160 g. powdered potassium bicarbonate, 100 g. potassium carbonate, and 66 g. potassium chloride in about 700 ml. of water in a liter volumetric flask; 100 ml. of a 4.4 per cent solution of

[199] *J. Assoc. Official Agr. Chem.*, **21**, 636 (1938).
[200] *J. Research Nat. Bur. Standards*, **24**, 241 (1940).
[201] *Biochem. Z.*, **2**, 271 (1907).
[202] *Biochem. Z.*, **49**, 1 (1913).

TABLE CXVI

REDUCTION FACTORS AND MOLECULAR REDUCING POWERS OF VARIOUS SUGARS FOR THE SCALES METHOD, AS MODIFIED BY ISBELL, PIGMAN, AND FRUSH

Sugar	Milligrams Anhydrous Sugar per Milliliter of 0.04 *N* Thiosulfate				Relative Molecular Reducing Power Glucose = 1
	5-ml. titer	10-ml. titer	15-ml. titer	20-ml. titer	
d-Glucose	1.078	1.092	1.107	1.127	1.00
d-Mannose	1.075	1.087	1.103	1.126	0.99
d-Fructose	1.040	1.050	1.078	1.097	1.01
l-Rhamnose	.....	0.973	0.999	.....	1.01
d-Galactose	1.215	1.223	1.240	1.267	0.89
d-Talose	.....	1.224	1.240	.....	0.89
l-Fucose	.....	1.201	1.235	.....	0.83
d-Gulose	.....	1.152	1.170	.....	0.94
l-Sorbose	.....	1.171	1.182	.....	0.93
l-Arabinose	1.078	1.092	1.107	1.127	0.83
d-Ribose	1.049	1.065	1.083	1.106	0.85
d-Xylose	0.996	1.007	1.021	1.040	0.91
d-Lyxose	.....	0.994	1.006	1.024	0.92
d-α-Galaheptose	.....	1.261	1.292	.....	0.99
d-β-Galaheptose	.....	1.259	1.283	.....	1.00
d-α-Glucoheptose	.....	1.291	1.313	.....	0.99
d-β-Glucoheptose	.....	1.318	1.341	.....	0.96
d-Glucoheptulose	.....	1.287	1.322	.....	0.97
d-α-Mannoheptose	.....	1.310	1.328	.....	0.96
d-β-Mannoheptose	.....	1.312	1.329	.....	0.96
d-α-Guloheptose	.....	1.291	1.312	.....	0.98
d-β-Guloheptose	.....	1.318	1.341	.....	0.96
4-β-Glucosidomannose	.....	.....	1.360	1.392	1.49
Cellobiose	.....	1.383	1.393	1.413	1.47
Maltose	1.479	1.471	1.485	1.497	1.39
Lactose	1.426	1.434	1.445	1.462	1.42
Lactulose	1.460	1.467	1.477	.....	1.39
Neolactose	1.427	1.433	1.440	1.451	1.44
Gentiobiose	.....	1.717	1.731	1.755	1.20
Melibiose	1.747	1.757	1.769	.....	1.18
Turanose	.....	2.691	2.714	.....	0.77
Invert Sugar	1.061	1.067	1.082	1.095	1.01

crystallized copper sulfate is added, and the volume is completed to 1 liter. Of this stock solution, 300 ml. is diluted with saturated potassium chloride solution to 1 liter, and this reagent is used for the sugar determination. The potassium chloride holds the reduced copper in solution so that it is unnecessary to add acid in order to dissolve the precipitated cuprous oxide, as in similar methods of this type. From 0.1 to 2 ml. of sugar solution, depending on the concentration, but con-

taining not more than 9 mg. of glucose, is mixed with 55 ml. of the copper-potassium chloride reagent in a flask with a flangeless narrow neck over which a short piece of rubber tubing is drawn so that about 2 cm. of it projects beyond the edge of the neck. The mixture is brought to the boiling point in $3\frac{1}{2}$ to $3\frac{3}{4}$ minutes, and then boiled for exactly 3 minutes. At the end of this time the open end of the rubber tube is closed with a pinchcock, to prevent entrance of air into the flask, and the flask is cooled under running water. The rubber tube is now removed and the solution titrated with standard iodine solution, using starch solution (1 g. starch dissolved in 100 ml. of saturated potassium chloride solution) as indicator, until the blue color does not fade in 10 seconds. The amount of iodine used is directly proportional to the milligrams of glucose throughout the range, 1 mg. glucose corresponding to 2.7 ml. $N/100$ iodine, 0.7 ml. $N/25$ iodine, or 0.285 ml. $N/10$ iodine. The equivalents for other sugars have not been determined, and the method, or later modifications of it, is used principally for the determination of glucose in blood and urine.

Shaffer and Somogyi's Micromethod for Reducing Sugars in Biological Fluids. Shaffer and Somogyi[203] made an extensive study to ascertain the most appropriate composition of the copper reagent, and the best possible conditions for measuring the reduced copper by the iodimetric method, without filtration of the precipitate. The older methods of Shaffer and Hartmann,[204] and of Somogyi[205] were modified accordingly. Shaffer and Somogyi's findings concerning the effect of the alkalinity of the copper reagent have been referred to on p. 787.

The reagent used contains both copper and iodine, the iodine in the form of iodate plus iodide. The iodine is liberated only after the solution has been acidified previous to titration with thiosulfate. The iodate in alkaline solution has no effect on the reducing sugar. Alkaline copper solutions in which iodide is incorporated are more stable, showing no autoreduction if 5 g. of potassium iodide per liter of solution is used. On the other hand, the iodide, like the chloride employed in Bang's later method, holds cuprous oxide in solution, and makes it sensitive to oxidation by air. For this reason the access of air must be prevented during the reduction and as long as the solution is alkaline. The addition of iodide has the further advantage that the copper reagent is less sensitive to reducing non-sugars present in biological materials.

The copper reagent is prepared as follows: 25 g. each of dry sodium

[203] *J. Biol. Chem.*, **100, 695 (1933).**
[204] *J. Biol. Chem.*, **45, 365 (1921).**
[205] *J. Biol. Chem.*, **70, 599 (1926); 100, 695 (1933).**

carbonate and of Rochelle salt is dissolved in about 500 ml. of distilled water in a beaker. Then 75 ml. of a solution containing 100 g. pure crystallized copper sulfate per liter is added with stirring from a pipette extending below the surface of the liquid. This is followed by the addition of 20 g. of dry sodium bicarbonate, which is dissolved with stirring, and of 5 g. of potassium iodide. The solution is washed into a 1-liter volumetric flask, 250 ml. of a 0.1 *N* solution of pure potassium iodate (3.567 g. dissolved to 1 liter) is added, and the solution is made up to the mark and mixed. It is filtered through a dry filter paper into a Pyrex bottle. If protected from strong light, the solution keeps unchanged for one to two years.

The iodate is used merely to release the necessary excess of iodine for the oxidation of the reduced copper. The quantity specified above permits the determination of 0.05 per cent glucose. If much less than this is present in the solution to be analyzed, the quantity of iodate solution used in making up the reagent may be correspondingly reduced, and time saved in the titration.

If slowly reacting sugars, such as xylose or mannose, are to be determined, the sodium bicarbonate is omitted and the alkalinity of the copper reagent increased by using 40 g. of sodium carbonate.

The thiosulfate solution for titrating back the iodine is prepared from an approximately 0.1 *N* stock solution, to 1 liter of which 10 ml. of 0.1 *N* sodium hydroxide has been added to render it more stable. The stock solution is diluted to 0.005 *N*, and standardized with 0.01 *N* potassium iodate solution, made by diluting the 0.1 *N* solution used in the preparation of the copper reagent.

Ten milliliters of the 0.01 *N* iodate solution, to which 1 ml. of *N* sulfuric acid and 2 ml. of 2.5 per cent potassium iodide solution have been added, should require exactly 20 ml. of the thiosulfate solution.

Five milliliters of the sugar solution to be analyzed, and containing not more than 2.5 mg. of glucose, is accurately measured into a Pyrex test tube (25 by 200 mm.), and 5 ml. of copper reagent is added from a pipette, the sugar solution being rinsed from the walls of the test tube. The tube is gently shaken to mix the contents and covered with a sealed glass bulb to prevent the access of air. A series of other tubes containing known amounts of glucose are prepared in the same manner, and also a blank, with 5 ml. water instead of sugar solution. The tubes are placed in a metal rack and immersed in a vigorously boiling water bath for 15 minutes. At the end of that time they are removed to a pan of cold water. When the solutions have cooled to about 30° C. add to each of the tubes 2 ml. of a solution containing 2.5 per cent each of potassium iodide and potassium oxalate, and then 5 ml. of *N* sulfuric

acid. (With copper reagents containing less than 150 ml. per liter of 0.1 *N* iodate, the addition of potassium iodide and oxalate is unnecessary.) The bulbs are replaced, and the tubes shaken to dissolve the cuprous oxide or iodide. The covered tubes are allowed to stand for 5 to 10 minutes to dissolve the precipitate completely. Then the bulbs and the walls of the tubes are rinsed, and the excess iodine is titrated with the 0.005 *N* thiosulfate, about 1 ml. of 1 per cent solution of soluble starch being used as indicator toward the end.

If less than 1 ml. thiosulfate is used for the titration, the result is doubtful because of approach to the capacity of the copper reagent. In such a case a more dilute sugar solution should be prepared. During the titration with thiosulfate a precipitate of copper oxalate may form, but this does not affect the results.

The titration value is deducted from the blank titration. The results of the tests with solutions of known sugar concentration are plotted on a curve, and the concentration of the unknown found from it. The following values given by Shaffer and Somogyi are shown as an example of the figures to be expected:

Mg. glucose in 5 ml. solution	2.00	1.00	0.50	0.25	0.10	0.05
Difference in thiosulfate used, ml	17.60	8.50	4.00	1.85	0.60	0.20

If the sugar solution to be analyzed contains less than 0.1 mg. of glucose in 5 ml., a copper reagent with 1 g. instead of 5 g. potassium iodide is to be preferred, because this gives a larger amount of cuprous oxide for equal quantities of sugar.

The method may also be used with sugars other than glucose, but, since the reducing power of different sugars varies, different heating periods are necessary. For glucose and arabinose 15 minutes is appropriate, for fructose 10 minutes, for xylose 30 minutes, for mannose 35 minutes, etc.

The Shaffer-Somogyi method has been recommended by Heinze and Murneek,[206] and also by Pickett[207] for the determination of sugars in plant materials.

When iodide is present in the copper reagent there is always some danger of reoxidation of the cuprous oxide dissolved by the iodide. Somogyi[208] found later that this oxidation can be prevented by the addition of sodium sulfate to the reagent. This is prepared by dissolving 25 g. of anhydrous sodium carbonate and 25 g. Rochelle salt in about 800 ml. of water. Add with stirring 40 ml. of a 10 per cent solution of

[206] *Missouri Agr. Expt. Sta., Research Bull.* 314 (1940).
[207] *J. Assoc. Official Agr. Chem.*, 23, 431 (1940).
[208] *J. Biol. Chem.*, 117, 771 (1937).

crystallized copper sulfate (4 g.), then 20 g. of sodium bicarbonate, 200 g. of anhydrous sodium sulfate, and 1.5 g. of potassium iodide. Heat to boiling, boil for half a minute, add 6 ml. of *N* potassium iodate solution, cool, and make up to 1 liter. The reducing-sugar determination is carried out as described above, the mixture of copper reagent and sugar solution being heated for 20 minutes in a boiling-water bath. The copper reagent is standardized with sugar solutions of known concentration. One-half milligram of glucose reduces copper equivalent to 4.6 ml. of 0.005 *N* thiosulfate.

In all methods where the reduced copper is determined directly by oxidation with iodine, a blank test should be run in the cold, to make allowance for substances like sulfur dioxide, which increase the result of the sugar determination. The iodine found in the blank is deducted from the iodine required in the test made at high temperature.

A number of methods have been devised in which the reduced copper is determined colorimetrically, without previous filtration. The cuprous oxide is dissolved in an excess of phosphomolybdic or phosphotungstic acid, and these are thereby reduced to lower oxides of an intense blue color. These procedures have been developed particularly for the analysis of blood and other biological fluids, by means of a microtechnique. The following method will serve as an example.

Benedict's Modification of the Folin and Wu Method.[209] The copper reagent used in this method contains citrate instead of tartrate. Jackson[210] has recommended the procedure for the determination of minute quantities of reducing sugars, in the neighborhood of 0.1 mg. per ml., which can be determined with a precision of about 1 per cent. The reagents are prepared as follows:

Copper Reagent: Dissolve 200 g. of sodium citrate and 60 g. of anhydrous sodium carbonate in about 800 ml. of water in a 1-liter volumetric flask. Dissolve 6.5 g. crystallized copper sulfate separately in about 100 ml. of water, and add this to the solution with agitation. Add 9 g. of ammonium chloride, dilute to 1 liter, and mix.

Tungstic Acid Reagent: Dissolve 100 g. of pure sodium tungstate in about 600 ml. of water in a liter flask. Add 50 g. of pure arsenic pentoxide, then 25 ml. of 85 per cent phosphoric acid and 20 ml. of concentrated hydrochloric acid. Boil for 20 minutes. After cooling, add 60 ml. of commercial formalin, 45 ml. of concentrated hydrochloric acid, and 40 g. of sodium chloride. Dilute to 1 liter and mix.

The reduction is carried out in a Folin and Wu sugar tube (Fig. 289). The bulb of this tube holds 4 ml., and above it is a constriction

[209] *J. Biol. Chem.*, 68, 759 (1926).
[210] *J. Assoc. Official Agr. Chem.*, 11, 175 (1928).

to prevent reoxidation of the cuprous oxide. Near the upper end of the tube is a mark indicating 25 ml. total volume. Two milliliters of sugar solution and 2 ml. of the copper reagent are pipetted into the tube, and in another tube are placed 2 ml. of standard glucose solution containing 0.1 (or 0.2) mg. per ml., and 2 ml. of copper reagent. The contents of the tubes are mixed by side-to-side shaking, and they are immersed in a boiling-water bath for 5 minutes. They are then cooled by placing in cold water, and 2 ml. of the tungstic acid reagent is added to each. After 1 to 2 minutes the volume is completed to 25 ml., and the solutions are compared by means of a colorimeter or photometer. The calculations are made in the usual way.

(*Courtesy of Eimer and Amend.*)

FIG. 289. Folin-Wu sugar tube.

Another modification of the Folin and Wu method has been developed by Werr[211] for the estimation of sugars in the different parts of the sugar-beet plant.

VOLUMETRIC REDUCTION METHODS BY MEANS OF MERCURY SOLUTIONS

Of other metallic salt solutions besides copper those of mercury have been used to some extent for determining reducing sugars.

Knapp's[212] Alkaline Mercuric Cyanide Method. The solution used in Knapp's method is prepared by dissolving 10 g. of pure mercuric cyanide and 100 ml. of sodium hydroxide solution of 1.145 sp. gr. to 1000 ml. The solution contains 7.9363 g. of metallic mercury per liter.

In making the determination a measured volume of the reagent, previously standardized against a known weight of the pure sugar, is heated to boiling and the sugar solution added from a burette until a drop of the filtered solution upon acidifying with acetic acid shows no coloration with ammonium sulfide solution. The calculation of sugar is made in the same manner as described under Soxhlet's volumetric method with Fehling's solution.

The end reaction in Knapp's method has been found uncertain, and the process is but little used.

Sachsse's[213] Alkaline Mercuric Iodide Method. The solution of Sachsse is prepared as follows: 18 g. pure dry mercuric iodide (prepared by precipitating mercuric chloride solution with potassium iodide, and washing and drying at 100° C.) is dissolved in a solution

211 *Z. Ver. deut. Zucker-Ind.*, **88, 155 (1938).**
212 *Ann.*, **154, 252 (1870).**
213 *Z. Ver. deut. Zucker-Ind.*, **26, 872 (1876).**

containing 25 g. of pure potassium iodide; a solution containing 80 g. of potassium hydroxide is then added and the volume completed to 1000 ml. The solution contains 7.9323 g. of metallic mercury per liter.

An alkaline stannous chloride solution, prepared by treating a solution of stannous chloride with an excess of potassium hydroxide, is used for determining the end point.

In making the determination a measured volume of reagent is heated to boiling, and the sugar solution added until a drop of the filtered solution shows no coloration with the alkaline tin solution. The comparative reducing power of several sugars upon Sachsse's solution is given in Table CXXVII (p. 970).

Sachsse's method has been variously modified, and two of these modifications will be described.

Method of Baudouin and Lewin for Small Quantities of Glucose.[214] The mercury reagent employed by these authors is prepared in two parts, like Soxhlet's solution. Solution A contains 3.6 g. mercuric iodide and 12 g. dry sodium iodide dissolved to a volume of 100 ml.; solution B is a *N* solution of sodium hydroxide prepared from metallic sodium and free from substances which react with iodine. To 15 ml. of the sugar solution which should contain not more than 4 mg. glucose is added 1 ml. each of solutions A and B, in a 50-ml. Erlenmeyer flask. The flask is stoppered with a cotton wad and placed for 3 minutes in a boiling-water bath. The flask is cooled, and 2 ml. of a solution of 1.783 g. chemically pure potassium iodate made up to 1 liter with 5 per cent sulfuric acid (by volume) is added. The 2 ml. of this standard solution, reacting with the sodium iodide in the mercury reagent, is equivalent to 10 ml. *N*/100 iodine. The metallic mercury is oxidized by the liberated iodine, and the excess of the iodine is titrated back with *N*/100 thiosulfate solution, using starch as indicator. The milligrams of glucose are directly proportional to the iodine used for oxidizing the metallic mercury. The number of milliliters of thiosulfate solution is subtracted from 10, and the difference, divided by 2.48, gives the milligrams of glucose.

Method of Fleury and Marque.[215] These authors have adapted Baudouin and Lewin's iodometric mercury method to the determination of larger quantities of glucose. Under these conditions the larger amounts of reduced mercury tend to conglomerate to metallic globules which do not readily dissolve, and finely divided barium sulfate is used to prevent this. The mercury reagent of Baudouin and Lewin is used. Forty milliliters each of solutions A and B, and 10 ml. of a

[214] *Bull. soc. chim. biol.*, 9, 280 (1927).

[215] *J. pharm. chim.*, [8], 10, 241, 292 (1929).

10 per cent suspension of precipitated barium sulfate (ground heavy spar is ineffective) is placed in a flask, the sugar solution is added, and the total volume made up to about 150 ml. with water. The flask is placed in a boiling-water bath for 6 minutes, and then cooled in running water. Then 20 ml. of 20 per cent sulfuric acid (by volume) is added and, after cooling the flask again, 25 ml. of *N*/10 iodine solution. When the mercury is completely dissolved the excess iodine is titrated back with *N*/10 thiosulfate solution. Each milliliter of *N*/10 iodine used for oxidizing the mercury is equivalent to 4.02 mg. glucose. As much as 100 mg. glucose can be determined by this method.

Fleury and Marque found that alkaline mercuric iodide solutions are reduced not only by reducing sugars but also by other polyhydroxy compounds, such as sugar alcohols, sugar acids, and non-reducing sugars, including polysaccharides. The reducing effect of these substances depends largely on the alkalinity of the mercury reagent and on the time of heating. In the use of these methods the chemist must assure himself that such other substances are absent, or that their quantity is not sufficient to affect the results.

Determination of Lactose in Milk by Fleury and Marque's Method.[216] Place 5 ml. of milk and about 30 ml. of water in a 50-ml. volumetric flask, and add drop by drop, with constant agitation, 1 ml. of mercuric nitrate solution which is prepared as follows. Transfer 105 ml. of concentrated nitric acid (sp. gr. 1.383), to a porcelain dish holding about 1 liter, and add gradually, with stirring, 145 g. of red mercuric oxide. When this is nearly dissolved, pour in 100 ml. of water, and heat to boiling. Cool the solution, place it in a 1-liter volumetric flask, fill with water to a volume of about 800 ml., add 35 ml. *N* sodium hydroxide solution, and fill to the mark. The mixture of milk, water, and mercuric nitrate reagent is filled up to 50 ml., and filtered. Ten milliliters of the filtrate, 30 ml. each of Baudouin and Lewin's solutions A and B, and 10 ml. of 10 per cent barium sulfate suspension are placed in a flask, and the mixture is diluted to about 100 ml. Place the flask in a boiling-water bath for 20 minutes, and then complete the determination as described above for glucose. One milliliter of *N*/10 iodine solution used to oxidize the reduced mercury is equivalent to 5.04 mg. lactose.

Hazard, Herbain, and Vaille[217] have applied the method of Fleury and Marque to the determination of galactose in urine in cases of galactosuria. One milliliter of 0.1 *N* iodine indicates 5.37 mg. galactose.

[216] *J. pharm. chim.*, [8], **10**, 292 (1929).
[217] *J. pharm. chim.*, [8], **21**, 61 (1935).

In making the determination, the reducing power of normal urine must be taken into consideration.

The mercury reduction methods have the advantage that the reduced metal is not reoxidized by the air, as is the case with cuprous oxide, that the reagent shows no autoreduction, and keeps well if stored in a dark bottle. Furthermore, within the ranges investigated, the quantity of reduced mercury is directly proportional to the quantity of reducing sugar present. Nevertheless, the high cost of mercury and of iodine has prevented them from coming into extensive use, except in combination with other reduction methods, for the analysis of sugar mixtures.

ESTIMATION OF HIGHER SACCHARIDES BY DETERMINING THE COPPER-REDUCING POWER AFTER HYDROLYSIS

The methods previously described in this chapter for determining reducing sugars are equally applicable to the analysis of the higher non-reducing saccharides provided the latter first undergo a quantitative hydrolysis into sugars of known reducing power.

The best examples of such applications of the method are the determinations of sucrose, starch, dextrin, and glycogen by means of Fehling's solution.

Determination of Sucrose by Means of Fehling's Solution

Sucrose upon treatment with invertase or acids is hydrolyzed quantitatively, 95 parts of sucrose yielding 100 parts of invert sugar. If the copper-reducing power of an inverted-sucrose solution is determined, the equivalent of invert sugar multiplied by the factor 0.95 will give the amount of sucrose present.

In making the determination care must be taken that the amount of sugar after inversion does not exceed the limit of the tables, which for 50 ml. of mixed Fehling's solution is about 240 mg. of invert sugar, or the equivalent of about 225 mg. of sucrose. The chemist should check the method with pure sucrose, in which case the following procedure may be employed.

Dissolve 1.9 g. of pure sucrose in about 75 ml. of water in a 500-ml. graduated flask and invert the solution according to the methods described in Chapter X. After cooling, the solution is nearly neutralized with sodium hydroxide (*carefully avoiding any excess*) and the volume completed to 500 ml.; 50 ml. of this solution (containing 200 mg. invert sugar = 190 mg. sucrose) is then treated according to any of the copper-reduction methods for invert sugar and the weight of reduced copper determined. The milligrams of invert sugar, corresponding to this weight

of copper, multiplied by the factor 0.95 gives the milligrams of sucrose.

In applying the method to the determination of sucrose in sugar-house products, and other substances which contain invert sugar, the difference between the invert sugar before and after inversion is multiplied by 0.95. The same method for determining invert sugar should be employed in both cases, and the proper correction for the reducing effect of the sucrose applied in calculating the invert sugar originally present. The method of calculation is best illustrated by an example:

Eight grams of a final cane molasses, known to contain approximately 50 per cent of total sugars was made up, after clarification, to 500 ml., and the invert sugar was determined by the method of Munson and Walker in 50 ml. of the solution, containing about 0.4 g. of total sugars. The result was 246.2 mg. copper, equivalent to 128.8 mg. invert sugar (column for 0.4 g. total sugars), or 16.10 per cent. For the second determination, 100 ml. of the molasses solution was transferred to a 250-ml. flask, inverted, neutralized, made to the mark, and 50 ml., containing 0.32 g. of molasses, was used. There was found 309.5 mg. copper, equivalent to 166.4 mg. invert sugar (column for invert sugar alone), or 52.00 per cent. The percentage of sucrose is then 0.95 (52.00 − 16.10) = 34.10.

If sucrose occurs in mixture with reducing sugars other than invert sugar the sucrose can be determined accurately only if the reducing effect of the sucrose in the presence of the other sugar is known. The Munson and Walker table has columns also for mixtures of sucrose and lactose; columns for sucrose plus glucose, and for sucrose plus fructose have been added by Erb and Zerban.[218] Corrections for the reducing effect of sucrose in mixtures with glucose or lactose have been determined for the method of Lane and Eynon (see p. 817).

If the sucrose is not in large excess over the reducing sugar, the correction for its reducing effect is generally small, and fairly accurate results may be obtained without applying the correction.

Example. Five grams of a sirup containing sucrose and maltose was made up to 500 ml. (solution A); 5 g. of the same sirup was dissolved, inverted, nearly neutralized, and made up to 500 ml. (solution B).

	Copper mg.	Invert Sugar mg.	Maltose mg.
50 ml. of sol. B gave by Munson and Walker's method	390	= 215.0	
50 ml. of sol. A gave by Munson and Walker's method	199	= 103.7	= 175.5
Difference	191	111.3.	

111.3 × 0.95 = 105.7 mg. = 21.14 per cent sucrose in sirup
175.5 mg. = 35.10 per cent maltose in sirup

[218] *Ind. Eng. Chem., Anal. Ed.*, **10**, 246 (1938).

Calculating the sucrose from the difference in copper, as is sometimes wrongly done, would give the following: 191 mg. copper = 99.3 mg. invert sugar (by Munson and Walker's table), 99.3 × 0.95 = 94.3 mg. = 18.86 per cent sucrose in sirup.

The unified methods and tables are most convenient for converting the equivalents of any reducing sugar into that of invert sugar. The same result, however, may be accomplished by means of the copper-reducing ratios given on p. 792.

Example. Ten grams of a sirup containing sucrose and fructose was made up to 500 ml. (solution A); 10 g. of the same sirup was dissolved, inverted, nearly neutralized, and made up to 500 ml. (solution B).

25 ml. of sol. B gave by Allihn's method 414 mg. Cu = 221 mg. glucose
25 ml. of sol. A gave by Allihn's method 195 mg. Cu = 100 mg. glucose

Difference = 121 mg. glucose

The reducing ratio of invert sugar to glucose is 0.958 for Allihn's method. 121 ÷ 0.958 = 126.3 mg. invert sugar; 126.3 × 0.95 = 120 mg. = 24.00 per cent sucrose in sirup.

The reducing ratio of fructose to glucose is 0.915 for Allihn's method. 100 ÷ 0.915 = 109.3 mg. = 21.86 per cent fructose in sirup.

Owing to the variation in the reducing ratios of sugars, especially in mixtures with other sugars, it is better to determine the equivalents by one of the unified methods.

Determination of Sucrose after Destruction of Reducing Sugars. When a sample contains only small quantities of sucrose, mixed with large amounts of several reducing sugars, the error in the determination of sucrose by the above method becomes greatly multiplied. For such cases Shapiro[219] recommends the destruction of the reducing sugars by means of alkali, prior to the determination of the reducing power of the original and the inverted solution. He proceeds as follows. Twenty milliliters of the solution which must not contain more than 120 mg. of reducing sugars is pipetted into a porcelain dish about 10 cm. in diameter, and 0.6 g. of sodium hydroxide is added in form of a solution. The total volume should be between 45 and 50 ml. The solution is well mixed with a glass rod and placed on a boiling-water bath for 25 minutes, with occasional stirring. The solution, which has become dark colored, is cooled and carefully neutralized against litmus with hydrochloric acid. It is then transferred to a volumetric flask,

[219] *Z. Ver. deut. Zucker-Ind.*, 86, 1 (1936).

clarified, made to the mark, and filtered, and the reducing power is determined in an aliquot by Bertrand's method (p. 801). Another aliquot is inverted by heating with 2 per cent hydrochloric acid for 5 minutes to 67–70° C., neutralized with sodium hydroxide, clarified, made to the mark, and filtered. The reducing power of this inverted solution is determined according to Bertrand, and the increase in the invert sugar found is multiplied by 0.95 to find the sucrose originally present. In mixtures of sucrose with glucose, fructose, and maltose, all four sugars being present in varying proportions, Shapiro found from 98.6 to 101.3 per cent of the sucrose taken.

Determination of Starch by Means of Copper Reagents

Starch upon heating with dilute hydrochloric acid is hydrolyzed almost quantitatively according to the equation $(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6$, in which 90 parts of starch yield 100 parts of glucose. The conversion of starch into glucose may be accomplished either by direct acid hydrolysis, as in Sachsse's method, or by first converting the starch into soluble products, as with diastase, and then hydrolyzing the filtered solution with acid.

Method of Sachsse, as Modified by the Association of Official Agricultural Chemists.[220] Stir a convenient quantity of the sample (representing from 2.5 to 3 g. of the dry material) in a beaker with 50 ml. of cold water for an hour. Transfer to a filter and wash with 250 ml. of cold water. Heat the insoluble residue for 2½ hours with 200 ml. of water and 20 ml. of hydrochloric acid (sp. gr. 1.125) in a flask provided with a reflux condenser. Cool, and nearly neutralize with sodium hydroxide; complete the volume to 250 ml., filter, and determine the glucose in an aliquot of the filtrate by any of the usual methods of copper reduction. The weight of glucose multiplied by 0.90 gives the weight of starch.

Owing to the fact that a perfect theoretical yield of glucose is never obtained from starch by acid hydrolysis, Ost[221] recommended the use of the factor 0.925 for converting glucose into starch by Sachsse's method.

Sachsse's method is one of the simplest processes for estimating starch, but has the objection of converting pentosans and other hemicelluloses into reducing sugars. The method for this reason gives too high results in the analysis of starchy substances which contain much cellular tissue. In order to eliminate this error the pentosans may be determined in a separate portion of the sample, as described in Chapter XV,

[220] "Methods of Analysis, A. O. A. C.," 5th ed., p. 359, 1940.

[221] *Chem. Ztg.*, 19, 1501 (1895).

and deducted from the starch found. But this correction is only approximate, because the composition of the pentosans varies, and the reducing power of the pentoses is not the same as that of glucose. It is therefore better to separate the starch from the cellular substances, by dissolving it directly, or by treatment with enzymes.

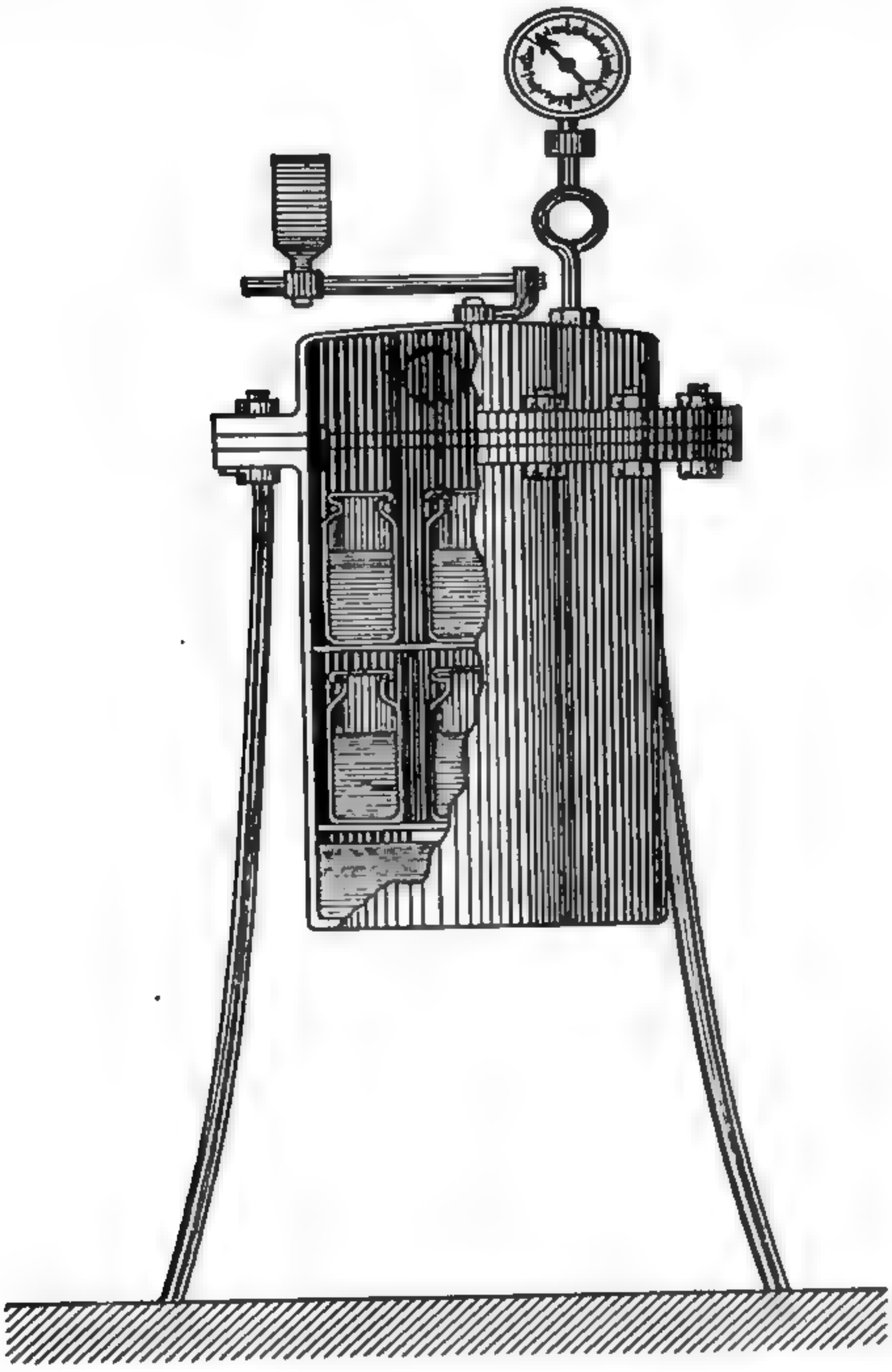


FIG. 290. Soxhlet's autoclave.

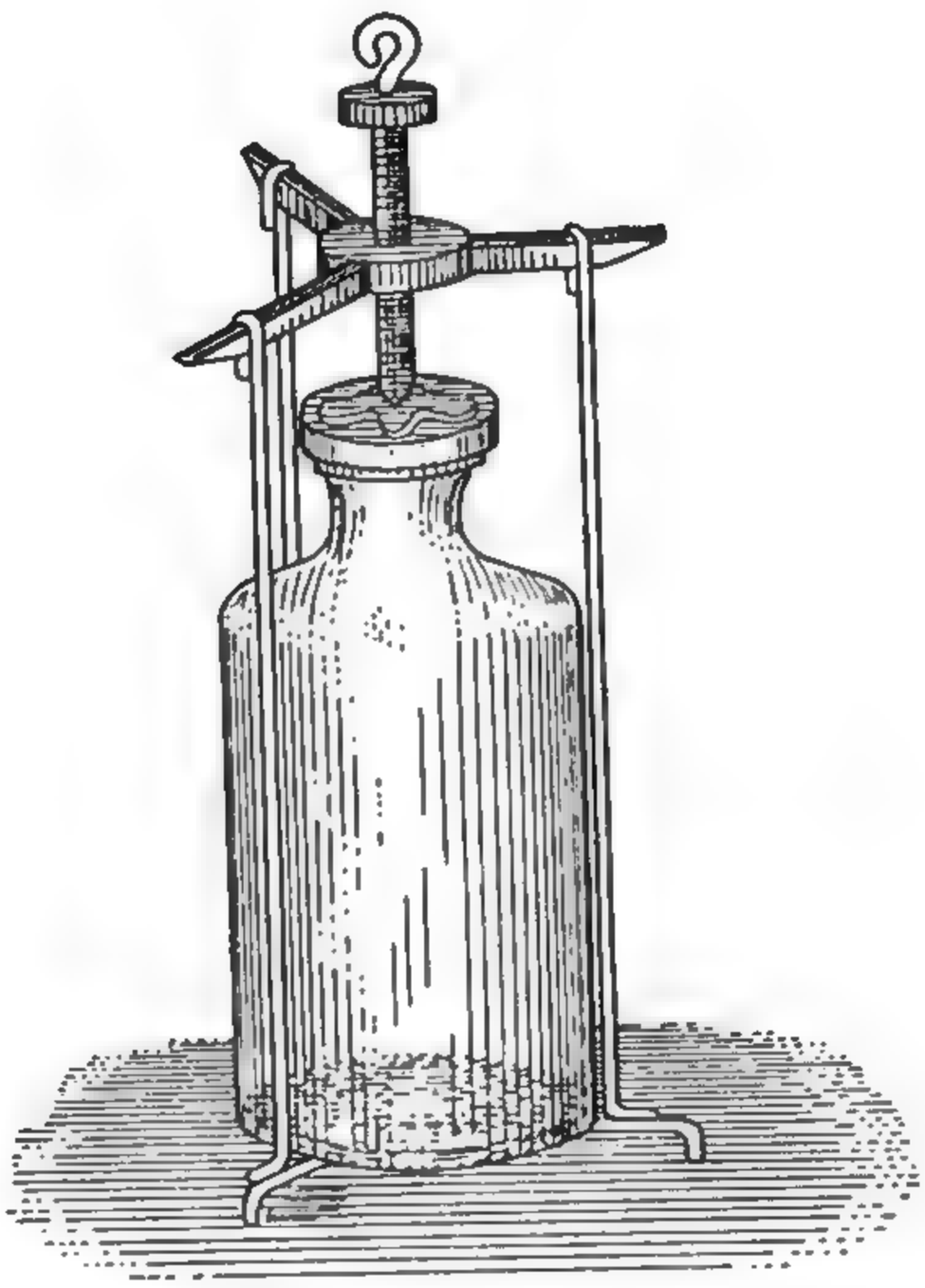


FIG. 291. Lintner's pressure bottle.

Method of Determining Starch by Solution under Pressure.[222] Three grams of the finely ground sample is extracted with cold water, as in the previous method, in order to remove sugars, dextrin, gums, etc. If much oil or fat is present the material should first be extracted with ether. The residue is then heated in a covered flask or metal beaker, of about 200-ml. capacity, with 100 ml. of water in an autoclave, a form of which designed by Soxhlet is shown in Fig. 290. The heating is continued for 3 to 4 hours at 3 atmospheres' pressure. If an autoclave is not available, Lintner pressure bottles (Fig. 291) may be used;

[222] König's "Untersuchung," 4th ed., Vol. III, 1, p. 439, 1910.

the bottles are immersed in a glycerol bath and heated for 8 hours at 108° to 109° C.

When the digestion is finished the pressure is first allowed to subside, when the autoclave or pressure flask is opened and the solution filtered through asbestos. The insoluble residue is well washed with hot water, and should show no blue reaction with iodine when examined under the microscope. The filtrate is made up to 200 ml. and then heated with 20 ml. of hydrochloric acid, of 1.125 sp. gr., for 3 hours in a boiling-water bath, the flask, which holds the solution, being connected with a reflux condenser. The solution, after cooling, is nearly neutralized with sodium hydroxide and made up to 500 ml. The copper-reducing power of the solution is then determined; the glucose equivalent of the copper multiplied by 0.9 gives the corresponding equivalent of starch.

Method of Determining Starch by Solution with Diastase. Märcker[223] found that the best method of dissolving starch from hemicelluloses was by means of diastase. The method of Märcker, as modified by the Association of Official Agricultural Chemists,[224] is as follows:

Preparation of Malt Extract. Use clean, new barley malt of known efficacy and grind only as needed. Grind well, but not so fine that filtration will be greatly retarded. Prepare an infusion of the freshly ground malt just before it is to be used. For every 80 ml. of the malt extract required digest 5 g. of the ground malt with 100 ml. of water, at room temperature, for 2 hours, or for 20 minutes if the mixture can be stirred by an electric mixer. Filter to obtain a clear extract. It may be necessary to return the first portions of the filtrate to the filter. Mix the infusion well.

Determination. Extract a convenient quantity of the substance (ground to an impalpable powder and representing from 4 to 5 gm. of the dry material) on a hardened filter with 5 successive portions of 10 ml. of ether; wash with 150 ml. of 10 per cent alcohol and then with a little strong alcohol. Place the residue in a beaker with 50 ml. of water, immerse the beaker in a boiling-water bath and stir constantly for 15 minutes or until all the starch is gelatinized; cool to 55° C., add 20 ml. of malt extract, and maintain at this temperature for an hour. Heat again to boiling for a few minutes, cool to 55° C., add 20 ml. of malt extract, and maintain at this temperature for 1 hour or until a microscopic examination of the residue with iodine shows no starch. Cool and make up directly to 250 ml.; filter. Place 200 ml. of the filtrate in a flask with 20 ml. of hydrochloric acid (sp. gr. 1.125); connect with a reflux condenser and heat in a boiling-water bath for 2½ hours. Cool, nearly neutralize with 10 per cent sodium hydroxide solution, finish the neutralization with sodium carbonate solution, and dilute to 500 ml. Mix the solu-

[223] " Handbuch der Spiritusfabrikation," p. 94, 1886.

[224] " Methods of Analysis, A. O. A. C.," 5th ed., pp. 359–360, 1940.

tion thoroughly, pour through a dry filter, and determine the glucose in an aliquot of the filtrate by any of the usual methods of copper reduction. Conduct a blank determination upon the same volume of the malt extract as used with the sample and correct the weight of glucose accordingly. The weight of the glucose obtained, multiplied by 0.90, gives the weight of starch.

Wein[225] has calculated a table for the above methods which gives the milligrams of starch or dextrin corresponding to milligrams of reduced copper as obtained by Allihn's method. The table was constructed by simply multiplying the milligrams of glucose in Allihn's table by the factor 0.9.

In some cases the correction for the sugars in the malt extract is larger than the starch content of the sample. In order to reduce this correction, various investigators have proposed the use of purified enzyme preparations, such as pancreatic amylase or takadiastase, instead of malt infusion. Fraps[226] has recommended takadiastase in the method described above. The results obtained by him with a number of feeding stuffs averaged somewhat lower than with malt extract, but were in some cases slightly higher.

Of the various processes for determining starch the diastase method secures the most perfect solution of starch with the least solution of accompanying hemicelluloses. However, if much cellular matter is present the hot water and malt solution may dissolve a small amount of pentosans which, by being afterwards hydrolyzed into reducing pentose sugars, introduce an error in the determination.

Diastase Method, in the Presence of Interfering Polysaccharides. The error just mentioned may be eliminated, according to Walton and Coe,[227] by precipitating the hemicelluloses with alcohol, after the treatment with malt infusion, and by additional modifications in Märcker's method. The Association of Official Agricultural Chemists gives the following directions for carrying out this procedure:[228]

Weigh 2–6 g. (charges of 4 g. for linseed meal, or 3 g. for dried apple pomace, have been found to be satisfactory) of the well-mixed sample, prepared to pass freely through a sieve not less than 40 mesh to the inch, using the smaller charges in the case of materials containing much gel-forming substance. (The weight of starch in the charge must not exceed 1.5 g.) Transfer to a dry 12.5–15-cm. close-textured rapid filtering paper in a glass funnel and extract with 5 successive portions of ether, taking for each portion more than enough to cover the charge and using a cover glass to retard evaporation.

[225] Wein's "Tabellen."
[226] *J. Assoc. Official Agr. Chem.*, 15, 304 (1932).
[227] *J. Agr. Research*, 23, 995 (1923).
[228] "Methods of Analysis, A. O. A. C.," 5th ed., pp. 360–361, 1940.

After completing the ether extraction, allow the ether to evaporate and then extract the charge with 300 ml. of dilute alcohol. The concentration of the alcohol may be varied somewhat to suit the material under examination. For linseed meal use 35 per cent alcohol (by volume) and for dried apple pomace use 25 per cent alcohol. Follow this with several filterfuls of 95 per cent alcohol and finish the leaching operations with a second ether extraction. Conduct also a control determination, preferably in duplicate, using a filter paper extracted with alcohol and the same quantity of water and malt extract as in the determination. (It is convenient to let the charge stand overnight at this point to allow the ether and alcohol to evaporate, as alcohol must be eliminated before starting the digestion with malt, or the charge may be dried at approximately 75° until the alcohol has been eliminated.)

Transfer as much of the dry material as possible from the filter paper into a glass mortar and pulverize all lumps. Transfer both filter paper and sample to a 500-ml. volumetric flask, add 20–30 ml. of water, and thoroughly wet the material by vigorous shaking.

Should more cold water be needed to make the material more fluid, calculate the quantity of hot water to be added accordingly, so that the total volume allowing for 40 ml. of malt solution will not exceed 200 ml. Let stand a few minutes and then add 100 ml. of actively boiling water. The sample now must be thoroughly gelatinized at boiling temperature in a water bath.

Cool to 50° or lower, add 20 ml. of malt extract[229] to controls as well as to charges, and place the flasks in a temperature-controlled water bath. Keeping the mash thoroughly mixed, gradually raise the temperature to 70° in 20–30 minutes. Maintain at 70° for 30 minutes, stirring the mixture from time to time, then increase the temperature to 80°, and keep it at that temperature for 10 minutes. Finally heat to the boiling point. Keep the mixtures well stirred. Cool the contents of the flasks and the water bath to 55°. Add 20 ml. of the malt extract, mix well, and hold at 55° for 1 hour, stirring about once every 10 minutes. At the termination of the digestion rapidly increase the temperature to above 80°.

Measure out 316 ml. of 95 per cent alcohol. Add a portion, a little at a time, to the contents of the flask, with thorough shaking between additions. After cooling to room temperature adjust the volume with water so that the quantity of liquid is 500 ml., making allowance for the volume occupied by the charge by adding 3 ml. of water for every 4 g. of charge present after bringing the contents to the 500-ml. mark. The determination may be interrupted at this stage for several days. The volume should be readjusted if evaporation has occurred in the meantime. Mix thoroughly, breaking up any ropy coagulum as much as possible by pouring back and forth from one large beaker to another. Filter through dry paper. Test the solid residue for starch, either microscopically or by the I color test, after elimination of alcohol and gelatinization with water. (If more than the merest trace of starch is found, reject the entire determination.) Evaporate exactly 200 ml. of the filtrate on a steam bath to a volume of 15–20 ml., or until practically all

[229] See p. 858 for the preparation of the malt extract.

alcohol has been expelled. Do not allow the evaporation to proceed to dryness.

Transfer the aqueous residue of starch conversion products to a 200-ml. volumetric flask with hot water, using a rubber-tipped rod to recover any dextrin that may be present. Allow to cool somewhat, and complete the volume to 200 ml. Transfer the contents to a suitable digestion flask, add 20 ml. of hydrochloric acid (sp. gr. 1.125) made by diluting 68 ml. of strong acid (sp. gr. 1.19, or 37 per cent hydrochloric acid) to 100 ml., and connect the flask with a reflux condenser. Heat in a boiling-water bath for 2.5 hours. Cool, and, for samples of linseed meal or other material yielding solutions which at this stage need further purification, add not more than 1 ml. of a 10 per cent solution of phosphotungstic acid in 1 per cent hydrochloric acid. Mix, and allow to stand at least 15 minutes. Increase the volume with water to 250 ml. in a volumetric flask, mix well, and filter through dry paper. Partially neutralize 200 ml. of the filtrate while stirring by adding 10 ml. of a strong solution of caustic soda (44 g. of sodium hydroxide per 100 ml. of water) and nearly complete the neutralization with a little powdered anhydrous sodium carbonate. Transfer to a 250-ml. flask with water, cool to room temperature, make up to the mark, and thoroughly mix. Filter, if necessary, and determine the glucose in a 50-ml. aliquot of the filtrate, gravimetrically. Correct the weight of glucose obtained by subtracting the weight of glucose found for the same aliquot of the malt control, and multiply the corrected weight of glucose by 0.90 to obtain the weight of starch.

It has been stated in connection with Sachsse's acid method of starch determination that the hydrolysis of starch is not complete. The same applies also to the hydrolysis of the maltose obtained by diastatic conversion. Experiments by W. A. Noyes[230] and his coworkers testing the action of 2.5 per cent hydrochloric acid upon the malt conversion of starch, show a hydrolysis into glucose which is about 97 per cent of the theoretical. A diminished yield of glucose necessitates the use of a conversion factor somewhat greater than 0.9, and Noyes recommends a factor of 0.93, for Märcker's method. Walton and Coe actually obtained by their procedure from 97.2 to 99.9 per cent of the starch added to linseed meal. It appears, therefore, that the error in the factor is partly compensated by other errors in the opposite direction.

Denny's Takadiastase Method. According to Davis and Daish,[231] and other investigators, takadiastase converts starch into a mixture of maltose and dextrose in varying proportions, depending on the exact conditions used. Collins[232] found, however, that if a sufficient quantity of diastase is used and the *p*H is adjusted to 5.0, the starch is converted

[230] *J. Am. Chem. Soc.*, **26**, 266 (1904).
[231] *J. Agr. Sci.*, **6**, 152 (1914).
[232] *Science*, **66**, 430 (1927).

completely into dextrose. Denny[233] confirmed these results, and has based on this a method which does not require subsequent hydrolysis with acid. A correction for the reducing substances present in the takadiastase is avoided by first dialyzing the solution of the enzyme preparation in a collodion sack overnight in running tap water. The volume of the solution increases during the dialysis, and account must be taken of this in measuring out the amount of solution required for the starch conversion. The dialyzed solution keeps well for several weeks at room temperature or lower.

If the powdered material contains no direct reducing substances a weighed sample is placed in a glass mortar and ground thoroughly with a little water. If there are reducing substances present the powder is first put on a filter paper in a funnel and is extracted with 50 per cent alcohol and then with ether. The powder is transferred with a spatula to the mortar, the filter paper being retained upon the funnel until the following day. Thorough grinding of the powder is necessary in order to obtain a negative test for starch on the residue at the end of the experiment. The thoroughly disintegrated powder is transferred to a large test tube with water to make a final volume of about 25 ml. The test tube containing the tissue is placed in a boiling-water bath for ½ hour. After cooling to room temperature, 25 ml. of 0.2 *M* acetic acid-acetate buffer at *p*H 4.5 is added and 25 ml. of a takadiastase solution which contains at least as much and preferably twice as much takadiastase as there are grams of starch in the sample. Toluene is added. The test tube is now placed in an incubator at about 38° C. until the following day, the tube being stoppered tightly and rotated end over end on a turning bar inside the incubator. The sample is filtered through the filter paper that has been retained from the previous day, into a volumetric flask, and a bit of the residue is examined with iodine microscopically for starch. If the test is positive the sample is again ground with a mortar and pestle and returned to the test tube, this time the filter paper itself being added. If the test is negative the filter paper and residue are added to the test tube. The sample is reincubated with takadiastase and buffer as before. The volumetric flask containing the filtrate is also placed in the incubator. On the following day, approximately 44 hours from the start, the digest is filtered into the volumetric flask, and the residue is washed repeatedly until the liquid in the flask occupies about four-fifths of the volume. Then saturated basic lead acetate solution is added until no further precipitate is obtained; it is made up to volume and deleaded with dibasic sodium phosphate. Aliquots are taken for the sugar determination and the glucose values are converted into starch by the use of the factor 0.93.

If experience with a given material has shown that the residue can be cleared of starch by a single grinding in the mortar the filter paper may be added to the digest at the start of the incubation, and the digestion may be continued without interruption until the end of the 44-hour period.

[233] *Contrib. Boyce Thompson Inst.*, **6**, 129, 381 (1934).

The results of this method checked well with those of the method of Walton and Coe (p. 859), except in the presence of inulin, which is not precipitated by the alcohol used in the latter method, and therefore causes high results. But with both these methods appreciable quantities of starch are found in some plant materials where it cannot be detected by a qualitative test, and there are indications that both malt diastase and takadiastase hydrolyze other plant constituents besides starch and also the interfering polysaccharides removed in Walton and Coe's method, with the formation of reducing substances. This subject is discussed further in Chapter XVII, pp. 1124–1131.

Method of Sullivan.[234] In order to separate the starch from other polysaccharides that may be hydrolyzed by either malt or takadiastase, Sullivan has devised a method in which the starch is first extracted with calcium chloride solution, precipitated with strong alcohol, redissolved in water, and reprecipitated with iodine and ammonium sulfate. The iodine-starch complex is heated with dilute hydrochloric acid, and the glucose determined by any of the usual methods. The details of the procedure are as follows:

Grind the dried plant material, from which the sugars need not be completely extracted, to pass through a 100-mesh sieve. Weigh out a sample containing about 100 mg. of starch into a 250-ml. Pyrex beaker that has been marked with a file at the 60-ml. volume. Add a few milliliters of water and stir the solution with a glass rod until all the particles are wet. Add 40–50 ml. of a saturated solution of calcium chloride of a specific gravity of about 1.48 and an alkalinity of about 0.025 *N*. If the calcium chloride is of less alkalinity, add to the sample 2 ml. of 0.1 *N* sodium hydroxide solution. Heat to boiling, and boil gently for 50–60 minutes. (A large hot plate will heat a dozen samples at a time.) Stir occasionally and add water to wash down the side of the beaker and to replace the water lost by evaporation. Do not allow the volume to become too low but keep at about 60 ml. When heating is complete, pour the mixture into a 100-ml. volumetric flask with a moderately wide neck, transferring all the insoluble particles into the flask with the use of a rubber policeman and hot water. Cool to room temperature, dilute to the mark with water, mix, pour into a dry centrifuge bottle, and centrifuge until the insoluble residue has settled. Decant the clear solution immediately into a dry beaker. If particles remain in suspension or float on the surface, pour the liquid through a coarse muslin cloth. Pipette an aliquot (50–75 ml.) of the solution into a 400-ml. beaker containing a volume of 95 per cent alcohol equal to 2.5 times the volume of the aliquot taken. Add about 0.5 g. of dry asbestos and stir well with a glass rod. Allow to stand overnight. Filter with suction through a Gooch crucible containing an asbestos mat and collect the starch precipitate and the asbestos on the mat. Rinse the beaker and the

[234] *J. Assoc. Official Agr. Chem.*, 18, 629–630 (1935).

contents of the crucible at least three times with 75 per cent alcohol. When the washing is complete, allow the liquid to be completely drained, removing as much alcohol as possible with the suction pump. Return all the asbestos and the starch precipitate back to the beaker and wash the inside of the crucible with a stream of hot water, catching the washings in the beaker. Add enough warm water to the beaker to make a volume of about 100 ml. and heat the mixture over a flame until it just reaches boiling. Stir continuously during the heating in order to avoid bumping. Cool to room temperature or lower. Add 1 ml. of a solution of 2 g. of iodine and 6 g. of potassium iodide in 100 ml. of water. Stir well. Add 50 ml. of a cold saturated solution of ammonium sulfate. Add water to bring the volume to about 200 ml. or to a volume that half fills the 400-ml. beaker. Stir well and allow the blue precipitate and the asbestos to settle. The supernatant liquid should be a clear amber color. If the blue color fails to settle, add more iodine and ammonium sulfate, stir, and allow to settle. After an hour filter with suction through a Gooch crucible containing a thin asbestos mat. Transfer the blue precipitate and the asbestos into the crucible and wash the beaker and the contents of the crucible several times with a solution composed of 1 part of saturated ammonium sulfate, 3 parts of water, and a few drops of iodine reagent. Wash three times with 75 per cent alcohol. Transfer the precipitate, now partly decolorized, and all the asbestos back into the beaker, using water. If the precipitate clings to the side of the crucible, place the crucible itself in the beaker. Add water to a volume of about 100 ml. and add 15 ml. of hydrochloric acid of a specific gravity of 1.1. Immerse the beaker in a boiling-water bath, stir until the blue color has disappeared, cover with a watch glass, and allow to digest 30 minutes. Remove from the water bath, filter off and wash the asbestos with warm water, and place the filtrate and washings in a 500-ml. short-neck Kjeldahl flask. (A sintered glass crucible is most convenient for this filtration.) Add glass beads and concentrate by boiling until the contents have reached a volume of about 100 ml. Connect with a reflux condenser and boil for an hour. Cool, nearly neutralize with sodium hydroxide solution, transfer to a volumetric flask (200 ml.), dilute to the mark, and determine the reducing power of an aliquot. Calculate the results as glucose, and multiply by the factor 0.9 to convert to starch. Correct for the volume of the non-starchy residue when the calcium chloride solution is made to volume.

In the analysis of a large number of plant materials, including wood, bark, roots, leaves, fruits, seeds, straw, flour, and bran, Sullivan obtained generally lower, and often much lower, results than by the takadiastase method, with or without subsequent acid hydrolysis. But the results are considered to be more accurate because of the removal of interfering substances.

The methods described here for the determination of starch require further modification in the analysis of materials other than cereal products, feed stuffs, and the usual plant materials. Fats occurring in

cacao products, coffee, etc., are first extracted with ether. Sugars contained in confectionery are removed by digestion with water. Meat products are first treated with alcoholic potash solution. Calcium salts in baking powders are dissolved out with cold, dilute hydrochloric acid. For the details of such pretreatments, and other modifications necessary in special cases, the chemist is referred to the "Methods of Analysis of the Association of Agricultural Chemists" and similar treatises.

Determination of Dextrin by Means of Copper Reagents

The principle of the method is the same as that described for starch. In the process described by König[235] a weighed amount of the dextrin is dissolved in cold water, made up to 1000 ml., and filtered. Three portions of 200 ml. each of the filtrate are heated in a boiling-water bath with 20 ml. of hydrochloric acid of 1.125 sp. gr. for periods of 1, 2, and 3 hours. The solutions are cooled, nearly neutralized with sodium hydroxide, and made up to volume so that the solution does not contain over 1 per cent glucose. The glucose is then determined by any of the usual methods, the highest results of the three experiments being taken as the correct value. The weight of glucose multiplied by the factor 0.9 gives the equivalent of dextrin.

If sugars are also present, the glucose equivalent of these must be subtracted from the glucose equivalent after hydrolysis and the difference calculated to dextrin.

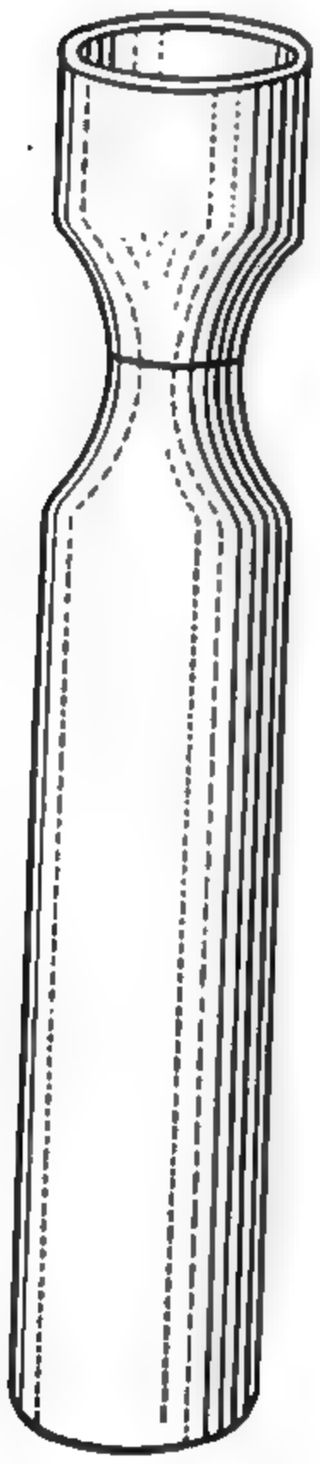
Determination of Dextrin in Beer. The above method is used by the Association of Official Agricultural Chemists for the determination of dextrin in beer.[236] Fifty milliliters of the beer, from which the carbon dioxide has been removed by vigorous shaking, is mixed with 15 ml. of dilute hydrochloric acid (sp. gr. 1.125), and the mixture is diluted to 200 ml. in a flask. A reflux condenser is attached, and the mixture is heated in a boiling-water bath for 2 hours. The solution is cooled and nearly neutralized with sodium hydroxide solution, the volume completed to 250 ml., and the solution filtered. The glucose is determined in an aliquot by any convenient method. In a separate portion of the beer the maltose is determined by one of the unified reducing sugar methods, for instance, Munson and Walker's, and the result, expressed as anhydrous maltose, is multiplied by 1.053, to convert to the glucose equivalent. This glucose is subtracted from the total glucose found, and the difference is multiplied by 0.9 to obtain the dextrin.

[235] König's "Untersuchung," 4th ed., Vol. III, 1, p. 427, 1910.
[236] "Methods of Analysis, A. O. A. C.," 5th ed., p. 152, 1940.

The hydrolysis of dextrin by dilute hydrochloric acid was found by W. A. Noyes[237] and his coworkers to be a little less than 95 per cent complete at the end of 2 hours' heating, and the results seemed to indicate that the theoretical yield of glucose could not be obtained even by prolonged heating. The theoretical factor 0.9 for converting glucose to dextrin is no doubt considerably too low for the method of acid hydrolysis.

Determination of the Total Invert Sugar Equivalent in Artificial Honey

In the manufacture of commercial invert sugar by heating highly concentrated sucrose solutions with very small amounts of acid to a high temperature, considerable quantities of reversion products are formed by condensation of the glucose and fructose. These products resemble dextrin, are precipitated by alcohol, and have a lower reducing power and levorotation than invert sugar. For this reason the estimation of the invert sugar present by the usual reduction methods does not give correct results. But it has been shown by Bruhns that these reversion products are more readily hydrolyzed by acids than the dextrins derived from starch. Artificial honeys usually contain only small amounts of sucrose, and these can be determined by hydrolysis with invertase, which does not hydrolyze the reversion products. The total carbohydrate content, that is the sum of sucrose, invert sugar, and reversion products, is according to Bruhns[238] determined as invert sugar and expressed as such.



(*Reproduced with permission from Frühling-Spengler, "Anleitung zu Untersuchungen," p. 131.*)

FIG. 292. Test tube for Bruhn's inversion method.

The method is similar to that employed by König for the estimation of dextrin (p. 865). Two and a half grams of the product is dissolved in a 250-ml. volumetric flask, 15 ml. of *N* hydrochloric acid is added, and the volume completed to the mark. Aliquots of this solution are transferred to eight special test tubes (Fig. 292) which have a constriction with a mark near the upper end, so that the water evaporated during the heating period may be replaced. The volume up to the mark is about 22 ml., permitting the withdrawal of two 10-ml. portions. The tubes are placed in a holder and immersed in a briskly

[237] *J. Am. Chem. Soc.*, **26**, 266 (1904).

[238] *Deut. Zuckerind.*, **58**, 939 (1933).

boiling, constant-level water bath. During the heating period the tubes are covered with glass caps or hollow glass spheres, to reduce evaporation to a minimum. The first tube is withdrawn after 2 hours' heating, and the others after subsequent heating periods of 1 to 2 hours each, over a total period of 12 hours. The tubes are rapidly cooled as soon as they are taken out of the bath, the volume is completed to the mark, and two 10-ml. portions are pipetted out. Each of the two portions is neutralized with sodium hydroxide and diluted to 20 ml. The invert sugar is determined by Bruhns's iodide-thiocyanate method (p. 833). The reducing sugar first increases with the time of heating, reaches a maximum, and then falls off again. The maximum figure is taken to represent the total invert sugar equivalent in the product. Bruhns found that, under the experimental conditions used, the glucose is perfectly stable, but the fructose is partially destroyed, and a correction must be applied. This is done by deducting from the thiosulfate titer of the sample, before deducting it from that of the blank, the milliliters of thiosulfate shown in the following table:

Heating period, hours	2	3	3.5	4	5	6	7
Deduct ml. 0.1387 *N* thiosulfate	0.12	0.24	0.29	0.34	0.40	0.45	0.50
Heating period, hours	8	9	10	11	12		
Deduct ml. 0.1387 *N* thiosulfate	0.55	0.60	0.65	0.70	0.75		

The maximum amount of invert sugar found, after application of this correction, represents the total carbohydrates in the artificial honey, expressed as invert sugar. Multiplication by 0.95 gives the quantity of sucrose used in the preparation of the artificial honey. If after 4 hours' heating the increase over the apparent invert sugar in the original sample exceeds 4 per cent, and it continues to increase upon prolonged heating, the presence of starch conversion products in the honey is indicated.

Determination of Glycogen by Means of Fehling's Solution

Trowbridge's Glycogen Method.[239] This method, which is a modification of Pflüger's[240] original procedure, is based upon the hydrolysis of the impure glycogen $(C_6H_{10}O_5)_n$, which has previously been precipitated from the solution of animal substance. According to the directions of the Association of Official Agricultural Chemists it is carried out as follows:[241]

[239] *J. Ind. Eng. Chem.*, 2, 21, 215 (1910).
[240] *Pflügers Archiv*, 114, 242 (1906).
[241] "Methods of Analysis, A. O. A. C.," 5th ed., p. 379, 1940.

Weigh by difference about 25 g. of the finely ground and thoroughly mixed sample. Place in a 400-ml. beaker and mix with 50 ml. of potassium hydroxide solution (1.5 + 1), free from carbonate. Cover the beaker with a watch glass and digest on a steam bath for 2 hours, stirring occasionally. At the end of the 2 hours, dilute to approximately 200 ml. with cold water.

Add to the solution an equal volume of 95 per cent alcohol, cover with a watch glass, and set aside for 10–12 hours. Decant the supernatant liquid through a folded 18.5-cm. filter, allowing the glycogen to remain in the beaker, and wash by decantation with 66 per cent alcohol (2 volumes of 95 per cent alcohol + 1 of water) until the glycogen is white, or nearly so. (Usually about 4 washings are required.) Transfer the washed precipitate from the beaker to the filter and wash 2 or 3 times with the 66 per cent alcohol. (The solution filters slowly, and the funnel should be covered with a watch glass to prevent excessive evaporation. The albuminous substance present retards the filtration if it is permitted to dry on the paper. If the washing by decantation is not made as complete as possible, it will be difficult to obtain the glycogen free from the coloring matter.)

After the washing is completed, close the bottom of the funnel by a piece of rubber tubing and a pinchcock. Fill the funnel with warm water, cover with the watch glass, and let stand 2–3 hours, or overnight. Open the pinchcock and allow all the solution to pass through the filter into a beaker. Close the funnel with the pinchcock and fill with warm water as before. Allow this water to remain in the funnel for 1 hour and then filter as before. At first the glycogen solution appears quite turbid. Continue washing with warm water until the filtrate becomes perfectly clear. To the solution of glycogen in water, add double its volume of 95 per cent alcohol and let stand overnight to complete the reprecipitation of the glycogen. Filter, and wash as before with 66 per cent alcohol.

If desired, the last filtration may be made through a weighed Gooch crucible and the weight of glycogen may be determined after drying to constant weight. This gives results that are approximately correct. More satisfactory results are obtained by hydrolyzing the glycogen with hydrochloric acid (1 + 3) and determining the resultant glucose. Dissolve the glycogen on the filter in warm water as directed above, collecting the filtrate and washings in a 300-ml. volumetric flask and keeping the volume within 225 ml. Add 12.5 ml. of hydrochloric acid to the combined filtrate and washings, mix, and place in a boiling-water bath for 3 hours. Cool, neutralize with 10 per cent sodium hydroxide solution, cool again, make up to volume with water, and determine glucose in an aliquot of the solution. The corresponding weight of glucose $\times$ 0.9 = its equivalent of glycogen. Correct this result for dilution to obtain the percentage of glycogen in the sample.

As in the case of starch and dextrin, the factor 0.9 is probably too low; Pflüger used the factor 0.927.

A more rapid method has been described by Good, Kramer, and Somogyi.[242] In a 15-ml. Pyrex test tube place 2 ml. of 30 per cent

[242] *J. Biol. Chem.*, **100**, 485 (1933).

potassium hydroxide for each gram of tissue to be analyzed. Stopper the test tube and weigh it. Add the tissue, submerge it, stopper and weigh again, to find the amount of tissue taken. Remove the stopper and place the tube in a boiling-water bath. When the tissue has dissolved, add 1.1 to 1.2 volumes of alcohol. Heat until the mixture begins to boil, cool, and centrifuge. Decant the supernatant liquid. Heat the residue in a boiling-water bath for a few minutes to expel the remaining alcohol, and then hydrolyze with 0.6 *N* hydrochloric acid, or normal sulfuric acid, by heating in a boiling-water bath for 2 to 2½ hours. Neutralize with sodium hydroxide, dilute to a definite volume, and determine the glucose by copper reduction. The glucose found is multiplied by 0.9 to convert into glycogen.

Determination of Fructosans by Copper Reduction

Kruisheer's Quantitative Levulosin Method for Detecting Artificial Honey.[243] Kruisheer found that certain genuine honeys give a positive reaction for hydroxymethylfurfural after being heated, and further that starch conversion products, especially unrefined starch sugar, which are sometimes mixed with honey, usually contain hydroxymethylfurfural. Under these conditions the usual method for detecting artificial honey cannot be employed, and for this reason Kruisheer has made use of an observation by Wohl,[244] who found that, when concentrated solutions of fructose are heated with very small quantities of acid, condensation takes place and a dextrinlike polysaccharide is formed which is insoluble in alcohol. Wohl named this substance levulosin. In dilute solution it is slowly hydrolyzed by acid to the original fructose. It is not fermented by yeast. The barium compound is soluble in 70 per cent alcohol. Levulosin does not occur in starch conversion products and is not formed in genuine honeys even after heating for 4 hours on the water bath, a much longer heating period being necessary before levulosin can be detected. Kruisheer's method is carried out as follows:

In the case of honey, 25 g. is boiled for a few minutes with 200 ml. of water in a 500-ml. Erlenmeyer flask stoppered with a cotton wad. The solution is cooled and inoculated with 15 g. pressed yeast which has previously been rubbed up with 20 ml. of sterile water. The mixture is fermented for 24 hours at low room temperature, being shaken carefully from time to time. It is then placed in an incubator at not over 30° C. for another 48 hours. When the fermentation is completed the mixture is centrifuged or filtered, and

[243] *Z. Untersuch. Lebensm.*, 63, 413 (1932).
[244] *Ber.*, 23, 2094 (1890).

neutralized with barium hydroxide, neutral red being used as indicator. The filtrate is concentrated on the water bath to 100 ml., and 200 ml. of 96 per cent alcohol and 50 ml. of saturated barium hydroxide solution are added. If the reaction is not alkaline to phenolphthalein, more barium hydroxide solution must be added. The mixture is well stirred and then allowed to stand for 24 hours, after which the precipitate is filtered off and washed with 70 per cent alcohol. The barium in the filtrate is precipitated with carbon dioxide. On the next day the barium carbonate is filtered off, and the alcohol is removed from the filtrate by distillation and evaporation to 25 ml. More water is added, and the solution again evaporated to 25 ml. in order to remove the last traces of alcohol. The solution is washed into a 110-ml. flask, diluted with water to the 110-ml. mark, and filtered if necessary. A few milliliters are used to determine the solids in the solution. Of the remainder, pipette 50 ml. each into two 100-ml. flasks, and add to each 5 ml. of 30 per cent sulfuric acid. Place both flasks in a water bath heated to such a point that the temperature within the flasks remains constant between 68° and 70° C. Heat one of the flasks to this temperature for 10 minutes, the other for 3 hours. The levulosin is completely hydrolyzed in the 3-hour period, but not to any extent in the first 10 minutes. Both solutions are cooled, neutralized against methyl orange, cooled again, and made up to the mark with water. An aliquot of each, containing not more than 0.45 g. solids, is diluted to 100 ml., and fructose is determined in both solutions according to the method of Kolthoff-Kruisheer (p. 902). The difference between the two fructose determinations gives the fructose which was present in the honey in the form of its condensation product levulosin. It is figured back to 100 parts honey.

Kruisheer found by this method from 0.50 to 2.51 per cent of levulosin, expressed as levulose, in various samples of artificial honey.

Artificial honey can be detected by this method also in baked products, such as honey cookies. The hydroxymethylfurfural is destroyed during the baking process, but not the levulosin, and no further levulosin is formed when the *p*H exceeds 6. Fifty grams of the cookie mass, from which the outer crust has been removed, is rubbed with luke-warm water to a homogeneous paste. This is washed into a 500-ml. flask, diluted to the mark, and the mixture centrifuged or filtered through a large filter. Four hundred milliliters of the filtrate is boiled for a few minutes in a large flask stoppered with a cotton wad. After cooling, 20 g. of yeast is added and the mixture is fermented for 3 days and further treated as described above.

According to Kruisheer,[245] levulosin is also formed from inulin in the roasting of chicory as a coffee substitute and can be detected by the same method as in artificial honey. Kruisheer claims that the inulin itself is completely hydrolyzed in the 10-minute treatment with

[245] *Z. Untersuch. Lebensm.*, **65, 275 (1933).**

acid. This is not correct, however, since Jackson and Goergen[246] have found that inulin always contains about 5 per cent of difructose anhydrides which are very resistant to hydrolysis.

Determination of Trifructosan in Flour according to Kruisheer.[247] This determination is based on Tillmans's[248] method for isolating trifructosan, followed by hydrolysis. Any sucrose present is precipitated and determined at the same time. Twelve and one-half grams of flour is shaken in an Erlenmeyer flask with 50 ml. of 70 per cent alcohol for 1 hour, and the mixture is filtered. Twenty-five milliliters of the filtrate is pipetted into a centrifuge tube and mixed well with 5 ml. of a normal solution of sodium hydroxide in 70 per cent alcohol (75 ml. of 96 per cent alcohol plus 25 ml. of 4 *N* sodium hydroxide). The tube is centrifuged the next day, the liquid decanted, and the residue washed twice with 2 ml. each of 70 per cent alcohol. The precipitate is dissolved in 10 ml. of warm water, and the solution neutralized with 0.25 *N* sulfuric acid, with methyl orange as indicator. The solution is washed into a 50-ml. flask with 15 ml. of water. Two and a half milliliters of 30 per cent hydrochloric acid (9.5 *N*) is added, and the flask is heated for 10 minutes to 68–70° C. The flask is cooled at once, the solution neutralized with sodium hydroxide, cooled again, and made to the mark. The total reducing sugars (*R*) are determined in 10 ml. of the solution by the method of Luff-Schoorl (p. 832), and the result calculated back to the weight of the flour taken. Another 20 ml. of the hydrolyzed solution is pipetted into a 50-ml. flask, and the fructose (*F*) is determined by the procedure of Kolthoff-Kruisheer (p. 902), but with half of all of the reagents used (2.5 ml. 4 *N* sodium hydroxide, 8 ml. iodine solution, etc.), and using 25 ml. of the total volume of 50 ml. for the copper reduction by the Luff-Schoorl method. The fructose thus found is calculated back to the weight of flour taken. Then the following formulas, which are self-explanatory, are applied:

$$\text{Glucose} = R - F$$

$$\text{Sucrose} = 2\,(R - F) \times 0.95$$

$$\text{Trifructosan} = 0.9\,[F - (R - F)] = 0.9\,(2\,F - R)$$

The results may be used to estimate approximately the percentage of rye and wheat flour in mixtures of the two, on the basis of the average trifructosan content of rye flour, *r*, and that of wheat flour, *w*. If the

[246] *Bur. Standards J. Research*, 3, 27 (1929).
[247] *Rec. trav. chim.*, 50, 153 (1931).
[248] *Z. Untersuch. Lebensm.*, 56, 26 (1928).

percentage of trifructosan of the mixture is called t, then

$$\text{Per cent rye flour in mixture} = \frac{100\,(t - w)}{r - w}$$

The trifructosan content of both rye and wheat flours varies within rather wide limits, depending on locality, variety, and methods of milling. For German conditions r is about 2.4, w about 0.32 per cent.

The Kruisheer method has been modified by Strohecker[249] to include the approximate estimation of rye flour in bread.

REDUCTION METHODS BY MEANS OF POTASSIUM FERRICYANIDE

The determination of glucose by means of potassium ferricyanide was first proposed by Gentele,[250] and interest in this reagent was revived by Hagedorn and Jensen's[251] work on blood-sugar estimation. The method is based on the reduction of ferricyanide to ferrocyanide in alkaline solution. Four molecules of ferricyanide furnish two atoms of oxygen, according to the equation:

$$4K_3Fe(CN)_6 + 4KOH \longrightarrow 4K_4Fe(CN)_6 + 2H_2O + O_2$$

The mechanism of this reaction has been studied by Wood[252] on the basis of oxidation-reduction potentials, with the following results:

1. Lowering of the alkalinity causes a slowing up of the reaction, and an increase in the final amount (A) of oxidant reduced, according to the equation $A = C - 1.5\ \Delta p\text{H}$, where C is a constant whose value depends on the particular conditions chosen.
2. A lowering of the temperature brings about the same qualitative result as a lowering of the alkalinity.
3. An increase in the salt content likewise retards the reaction and increases the final quantity of oxidant reduced.
4. The final quantity of oxidant reduced is directly proportional to the concentration of the sugar, within the limits of blood-sugar determinations.
5. Increasing the concentration of the oxidant slightly increases the final amount of oxidant reduced.
6. The reducing effect of glucose is greatly increased by the presence of sodium cyanide.

These findings are similar to those obtained in the study of alkaline copper solutions and discussed on pp. 787 and 821.

[249] *Z. Untersuch. Lebensm.*, **63**, 514 (1932).
[250] *Chem. Zentr.*, **1861**, I, 91; *Z. anal. Chem.*, **9**, 453 (1870).
[251] *Ugesk. Laeger*, **80**, 1217 (1918); *Biochem. Z.*, **135**, 46 (1923).
[252] *J. Biol. Chem.* **110**, 219 (1935).

Ionescu and Vargolici's Direct Titration Method.[253] This is carried out similarly to Soxhlet's volumetric method. The reagent is prepared by dissolving 46 g. potassium ferricyanide and 46 g. potassium hydroxide to a total volume of 1 liter. Ten milliliters of the reagent and 20 ml. of water are transferred to an Erlenmeyer, and 10 drops of a 1 per cent solution of picric acid is added to serve as indicator of the end point. The reagent is standardized by heating to boiling and running in from a burette a solution containing 5 g. of glucose in 1 liter. The solution gradually becomes lighter in color, but as soon as all the ferricyanide is reduced the liquid suddenly turns cherry red, the picric acid being reduced to picramic acid. Methylene blue may also be used as an internal indicator, as in the method of Lane and Eynon. Exactly 10 ml. of standard glucose solution should be required to reduce 10 ml. of ferricyanide reagent. The determination of glucose is carried out in exactly the same manner as the standardization, and the calculation is made as in Soxhlet's method, the volume of sugar solution being inversely proportional to its glucose content. Ten milliliters of the ferricyanide solution corresponds to 50.0 mg. of glucose, 49.1 mg. of invert sugar, 63.7 mg. of maltose, and 67.6 mg. of lactose.

The sugar solution to be tested may contain as much as 30 per cent of sucrose in the presence of 0.5 per cent of glucose without the result being affected.

This method has the advantages that the reagent is easily prepared, is cheap, and keeps well in a bottle of dark-colored glass, and no precipitate is formed to obscure the end point. It is well adapted for routine work in the sugar factory.[254]

Hanes's Ferricyanide Micromethod.[255] The method of Hagedorn and Jensen, referred to above, permits the determination of only 0.385 mg. glucose as the maximum. Hanes's modification extends this limit to about 4 mg. An excess of ferricyanide is used, and the unreduced ferricyanide is measured iodometrically. The following reagents are used: The ferricyanide solution contains 8.25 g. potassium ferricyanide and 10.6 g. anhydrous sodium carbonate to the liter. The solution is kept in a dark bottle and is not used until 2 or 3 days after its preparation; from then on the titer remains constant. The iodide solution is prepared by dissolving 12.5 g. potassium iodide, 25.0 g. zinc sulfate, and 125 g. sodium chloride to a total volume of 500 ml. This solution is filtered through a double filter paper. The zinc sulfate precipitates

[253] *Bull. soc. chim. Romania,* **2,** 38 (1920); Hamy, *Bull. assoc. chim. sucr. dist.,* **47,** 385 (1930).

[254] Rover, *Bull. assoc. chim. sucr. dist.,* **49,** 421 (1932).

[255] *Biochem. J.,* **23,** 99 (1929).

the reduced ferrocyanide as zinc salt, while the potassium iodide is oxidized by the excess ferricyanide to iodine which is measured with thiosulfate solution. To prepare the starch solution used as an indicator, 1 g. of soluble starch is shaken up with 20 ml. of cold water, and the mixture poured into 60 ml. boiling water. Boiling is continued for 2 minutes, 20 g. of sodium chloride is added, the solution is cooled and made up to 100 ml.

The reduction tests are carried out in test tubes about 1 inch wide and 7 inches long, and they are covered with glass bulbs during the reaction. A number of tests can be run at one time. Five milliliters of the ferricyanide reagent is measured into each tube, and 5 ml. of sugar solution is added. The tubes are heated for 15 minutes in a boiling-water bath, immersed about 2 to 3 inches. They are then cooled in running water for about 3 minutes, 5 ml. of the iodide-zinc solution is added, then 3 ml. of acetic acid (5 ml. glacial acetic acid diluted to 100 ml.), and the liberated iodine is at once titrated in the same tube with $N/100$ thiosulfate from a 10-ml. microburette with 0.02-ml. divisions. When the color of the solution has become pale yellow, starch indicator is added and the titration completed. A blank is run with 5 ml. of water. The milliliters of thiosulfate used in the blank, less the milliliters used in the determination, are equivalent to the amount of ferricyanide reduced, and thus to the amount of reducing sugar present.

Hanes used only glucose and maltose in his work, but Sobotka and Reiner[256] experimented with eleven sugars, and their results are shown in Table CXVII.

Values intermediate between those given in the table may be found by drawing curves based on the experimental results of Sobotka and Reiner, and interpolating.

Hanes claims as a special advantage of the ferricyanide method that, unlike cuprous oxide, the ferrocyanide formed is not reoxidized by air. The method has been used by Blackwood[257] for the determination of lactose in deproteinized milk, with satisfactory results.

Hulme and Narain's Modification of Hanes's Method.[258] It is noted from Table CXVII that there is no strict linear relationship between milligrams of reducing sugar and milliliters of thiosulfate. Hulme and Narain found, however, that by simply changing the volume of sugar solution from 5 ml. to 10 ml., and operating otherwise exactly according to Hanes's directions, a straight-line relationship is

[256] *Biochem. J.*, **24**, 394 (1930).
[257] *J. Dairy Research*, **5**, 245 (1934).
[258] *Biochem. J.*, **25**, 1051 (1931).

TABLE CXVII

	Milligrams Reducing Sugar								
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
	Milliliters $N/100$ Thiosulfate Solution								
Glucose	1.45	2.91	4.40	5.91	7.38	8.93	10.55	.....	.....
Fructose	1.55	3.10	4.70	6.28	7.83	9.39	10.90	12.48	.....
Invert Sugar, as sucrose	....	2.88	4.34	5.87	7.32	8.87	10.36	11.91	.....
Galactose	1.02	2.19	3.40	4.50	5.64	6.86	8.12	9.12	10.41
Mannose	1.45	2.92	4.30	5.87	7.46	8.63	10.38	11.42	.....
Maltose	1.33	2.44	3.63	4.96	6.08	7.23	8.47	.....	.....
Lactose	1.22	2.44	3.49	4.54	5.78	6.89	7.92	8.88	9.92
Xylose	1.55	3.11	4.65	6.07	7.49	9.06	10.59	11.83	.....
Arabinose	1.40	2.72	4.15	5.55	7.05	8.41	9.92	11.28	12.29
Ribose	1.25	2.55	3.79	5.11	6.39	7.76	9.14	10.49	11.76
Rhamnose	1.63	3.13	4.86	6.49	7.67	9.27	10.51	11.56	12.10

obtained. Under these conditions the milligrams of reducing sugar equal $b \times$ (ml. $N/100$ thiosulfate $+ 0.05$). The factor b equals 0.340 for glucose, 0.341 for fructose, 0.455 for maltose. For invert sugar prepared from sucrose by acid hydrolysis Hulme and Narain found a factor of 0.338. But this is evidently due to the formation of reversion products during the hydrolysis, because an equimolecular mixture of glucose and fructose gave a factor midway between the values for the constituents of the mixture, and the factor was generally found to be additive.

Many other modifications of the Hagedorn and Jensen method have been proposed. They differ from the original procedure principally in the composition of the ferricyanide reagent and in the method for determining the quantity of ferricyanide reduced to ferrocyanide. Only some of these modifications can be mentioned here, and for the details the chemist is referred to the original literature.

Van Slyke and Hawkins[259] allow the excess ferricyanide to react with hydrazine and measure the nitrogen gas evolved, by means of the Van Slyke-Neill[260] manometric apparatus. Hawkins[261] has found that the amount of nitrogen is directly proportional to the weight of glucose, mannose, maltose, or lactose; the same is true of fructose, arabinose, and xylose, but only up to a concentration of 0.1 mg. sugar per milliliter solution. The reducing ratios of mannose, xylose, and lactose, based on the reducing power of glucose, are about the same as in the Bertrand method, but this does not hold for the other sugars mentioned.

[259] *J. Biol. Chem.*, **79**, 739 (1928).
[260] *J. Biol. Chem.*, **61**, 523 (1924).
[261] *J. Biol. Chem.*, **84**, 79 (1929).

In Whitmoyer's micromethod[262] the reduced ferrocyanide is directly titrated with a standard solution of ceric sulfate, Alphazurine G being used as an internal indicator. Another procedure, based on the same principle but with orthophenanthroline as indicator, and permitting the determination of as much as 10 mg. of reducing sugar, has been described by Hassid.[263] Miller and Van Slyke,[264] and also Hassid,[265] later recommended Setopaline C, a technical product of secret composition, as a more sensitive indicator for titration with ceric sulfate.

If the sugar solution is colorless, the amount of ferricyanide reduced can be determined colorimetrically or photometrically by the diminution of the color of the solution, as suggested by Hoffman.[266] According to Folin,[267] the ferrocyanide obtained by reduction may be treated with ferric sulfate, and the Prussian blue formed determined colorimetrically.

In still another modification, Hawkins[268] measures the time necessary for the complete reduction of a given quantity of ferricyanide, as is done in the methylene blue method of Baerts and Binard (see p. 877).

An application of the ferricyanide method to the determination of diastatic power is described in Chapter XVII (p. 1142).

Strepkov[269] observed that under certain conditions alkaline ferricyanide oxidizes fructose selectively. In a further study of this subject Englis and Becker[270] found that a reagent containing 4 g. potassium ferricyanide, 150 g. of anhydrous sodium carbonate, and 250 g. of crystallized disodium phosphate per liter oxidizes about eighteen times as much fructose as it does glucose, in 15 to 30 minutes at 50° C. The carbonate increases the rate of oxidation of both sugars, while the phosphate decreases it but has a greater retarding action on glucose than it has on fructose.

A micromethod in which the amount of ferricyanide reduced and the corresponding quantity of reducing sugar is determined by measuring the oxidation-reduction potential at the end of the reaction has been described by Shaffer and Williams.[271] It has been modified, and applied to the determination of invert sugar in raw and refinery products

[262] *Ind. Eng. Chem., Anal. Ed.*, **6**, 268 (1934).
[263] *Ind. Eng. Chem., Anal. Ed.*, **8**, 138 (1936).
[264] *J. Biol. Chem.*, **114**, 583 (1936).
[265] *Ind. Eng. Chem., Anal. Ed.*, **9**, 228 (1937).
[266] *J. Biol. Chem.*, **120**, 51 (1937); see also Forsee, *Ind. Eng. Chem., Anal. Ed.*, **10**, 411 (1938).
[267] *J. Biol. Chem.*, **77**, 421 (1928); **81**, 231 (1929).
[268] *J. Biol. Chem.*, **81**, 459 (1929); **84**, 69 (1929).
[269] *Biochem. Z.*, **287**, 33 (1936).
[270] *Ind. Eng. Chem., Anal. Ed.*, **11**, 145 (1939); see also *ibid.*, **13**, 15 (1941).
[271] *J. Biol. Chem.*, **111**, 707 (1935).

by de Whalley.[272] Its reliability and its practical advantages over the usual methods remain to be demonstrated.

According to Heinze and Murneek,[273] and also Pickett,[274] alkaline ferricyanide reagents are reduced by various non-sugars, and the results obtained with them usually give higher sugar values than those found by means of copper reagents.

REDUCTION METHODS BY MEANS OF METHYLENE BLUE

Methylene blue has already been mentioned as an internal indicator in reduction methods with copper reagents and with potassium ferricyanide. Its use there is based on the reduction to the leuco compound by the slightest excess of reducing sugar; and this property may be utilized also directly for the estimation of these sugars. Wohl[275] first recommended it for qualitative purposes, and Pavlas[276] has published a procedure for the approximate determination of invert sugar in beet sugars. In this method Pavlas also made use of a new principle, suggested by Staněk.[277] Instead of measuring the quantity of one of the chemicals entering into the reaction, as is usually done, this author determined the time necessary for the complete reduction of a given amount of oxidizing agent. The same principle is applied in the following quantitative method.

Baerts and Binard's[278] Method for Determining Invert Sugar in Beet and Refined Cane Sugars. These authors use a 1 per cent solution of methylene blue (Grübler's medicinal grade), and Soxhlet's alkaline Rochelle salt solution. Ten grams of the sugar to be analyzed is dissolved in water in a Pyrex Erlenmeyer flask of 250-ml. capacity; 0.5 ml. each of methylene blue solution and of Rochelle salt solution is added, and the mixture is diluted to 50 ml. In order to prevent evaporation, and at the same time to exclude the air as far as necessary, the flask is provided with a straight Liebig reflux condenser. The flask is placed on a metal gauze, and the burner is regulated so that the solution begins to boil in 2 minutes to 2 minutes and 10 seconds. As soon as the boiling extends through the entire liquid a stop watch is started. At the moment when the color of the methylene blue is completely discharged, the elapsed time is read. In routine work care

[272] *Intern. Sugar J.*, 41, 312 (1939).
[273] *Missouri Agr. Expt. Sta., Research Bull.* 314, 1940.
[274] *J. Assoc. Official Agr. Chem.*, 23, 431 (1940).
[275] *Z. Ver. deut. Zucker-Ind.*, 38, 347 (1888).
[276] *Z. Zuckerind. čechoslovak. Rep.*, 57, 272 (1932/33).
[277] *Z. Zuckerind. čechoslovak. Rep.*, 56, 41 (1931/32).
[278] *Sucr. belge*, 52, 317 (1933).

must be taken that all the flasks used have about the same dimensions and weight. The light must fall on the flask in such a way, and the angle of observation must be such, that the disappearance of the blue color can be accurately judged. The milligrams of invert sugar corresponding to the time necessary for complete decolorization of the methylene blue are found from Table CXVIII.

TABLE CXVIII

Milligrams Invert Sugar in 10 g. Sugar	Time Necessary for Complete Decolorization of Methylene Blue	
	min.	sec.
0	36	00
1	18	00
2	9	30
3	5	12
4	3	12
5	2	22
6	1	51
7	1	33
8	1	13
9	1	04
10	0	58
15	0	37
20	0	23
50	0	14

This method gives the most reliable results in the range of 0 to 0.1 per cent invert sugar, where the Herzfeld method lacks precision.

Method of de Whalley for High-Grade Refined Sugars.[279] This method covers a range up to 0.015 per cent invert sugar. The partial decolorization of a 0.2 per cent methylene blue solution (British Drug Houses technical grade) is used as the criterion. Seven grams of the ground sugar sample is weighed into a clean, dry test tube of white glass, 6 inches long by 0.75 inch in diameter. All the tubes used for the test and for the standards should have about the same weight. Those used for the tests are fitted with large rubber rings around the top so that they can be supported in a constant-level boiling-water bath. This is made of sheet copper, 7 inches cube, with three holes in the top. The center hole is for the test tube, the other two serving as steam vents. The level of the water in the bath is 2 inches below the top. The bath is heated with a ring burner, supplied by gas at a constant pressure of 3.5 to 3.75 inches of water and maintained by a pressure regulator.

[279] *Intern. Sugar J.*, **39, 300 (1937).**

The 7-g. sample of sugar is dissolved in 6 ml. of water, 1 ml. of methylene blue solution, and 1 ml. of 3 *N* sodium hydroxide solution, the last two measured with a microburette. The tube is stoppered with a cork, shaken vigorously for 15 seconds, and immersed in the boiling-water bath for exactly 120 seconds. It is then removed, and the color is compared within 5 seconds with a row of standard color tubes, containing copper sulfate and excess ammonia. The copper sulfate solution is prepared by dissolving 19.5 g. of the crystallized salt to a total volume of 500 ml. in boiled distilled water; the ammonia is of 0.880 specific gravity and contains 32.9 per cent ammonium hydroxide by titration. The copper sulfate solution is mixed with the ammonia in the proportions shown in Table CXIX. Each mixture is made up to

TABLE CXIX

STANDARDS FOR DE WHALLEY'S INVERT-SUGAR METHOD

Per Cent Invert Sugar, Standard	Copper Solution	Ammonia
	milliliters in 50 ml. standard	milliliters in 50 ml. standard
0.001	40.00	10
0.002	24.60	10
0.003	16.40	10
0.004	10.66	10
0.005	7.18	10
0.006	4.92	10
0.007	2.97	10
0.008	2.26	10
0.009	1.74	10
0.010	1.33	10
0.015	0.50	10

50 ml. total volume and sealed in a test tube of the dimensions given above. The table also shows the percentage of invert sugar corresponding to each standard under the experimental conditions specified.

Sugars containing more than 0.015 per cent invert sugar are first mixed with invert-free sucrose in known proportions so that the total weight equals 7 g., and the invert sugar found is corrected for the dilution.

This method is more rapid and convenient than Main's pot method (p. 818), and also more rapid than that of Baerts and Binard when the invert-sugar content is low.

In a number of refined-sugar samples de Whalley obtained good checks with Main's pot method.

REDUCTION METHODS USING NITROPHENOLS

The reduction of nitrophenols to deeply colored aminophenols forms the basis of various procedures for determining reducing sugars. Thus picric acid (trinitrophenol, $C_6H_2(NO_2)_3OH$) is converted into dark red picramic acid, $C_6H_2(NO_2)_2NH_2OH$, when heated in alkaline solution with glucose. This reaction was first observed by Braun,[280] and has been applied to the determination of glucose in blood by Lewis and Benedict.[281] As an example of picric acid methods that of Thomas and Dutcher is described.

Thomas and Dutcher's Method for Determining Reducing Sugars.[282] In this procedure the reagent of Benedict and Osterberg[283] is employed, which is prepared as follows. Thirty-six grams of picric acid which has been recrystallized from dilute hydrochloric acid and dried at 60° C. is added to 500 ml. of 1 per cent sodium hydroxide solution in a 1-liter volumetric flask; 400 ml. of hot water is added, the mixture is shaken until all the picric acid is dissolved, and after cooling the volume is made up to the mark. Ten milliliters of the sugar solution to be tested, containing from 0.01 to 0.07 per cent reducing sugar, is pipetted into a Pyrex test tube holding about 50 ml. Ten milliliters of the picric acid reagent is added, and then 2 ml. of 25 per cent sodium carbonate solution. The total volume must always be 22 ml. Another tube is prepared in exactly the same way, with 10 ml. of a standard glucose solution of 0.025 per cent strength. The tubes are plugged with a cotton wad, placed for 20 minutes in a water bath heated to 95° C., and then cooled to room temperature in running water. Both standard and test solution are suitably diluted and compared in a colorimeter.

It was found by Thomas and Dutcher that 0.99 part of fructose, 1.01 of arabinose, and 1.08 of xylose have the same reducing effect on the picric acid reagent as 1 part of glucose. Sucrose up to a concentration of 1 *M* has no reducing effect, but it can be determined after previous hydrolysis. The method has been applied by Thomas[284] to the determination of starch, hydrolysis being effected with takadiastase. A similar colorimetric procedure for determining reducing sugars and starch by means of picric acid has been described by Coe and Bidwell.[285]

Willaman and Davison[286] have called attention to the fact that the

[280] *Z. anal. Chem.*, **4**, 185 (1865).
[281] *J. Biol. Chem.*, **20**, 61 (1915).
[282] *J. Am. Chem. Soc.*, **46**, 1662 (1924).
[283] *J. Biol. Chem.*, **34**, 195 (1918).
[284] *J. Am. Chem. Soc.*, **46**, 1670 (1924).
[285] *J. Assoc. Official Agr. Chem.*, **7**, 297 (1923/24).
[286] *J. Agr. Research*, **28**, 479 (1924).

color depth of picramic acid solutions is not strictly proportional to the concentration, and that for this reason the standard should always be of such concentration as to match the unknown as closely as possible.

F. Herzfeld[287] tested the picrate method of Benedict and Osterberg with solutions of raw sugars and molasses. Although the picramic acid concentration was determined with the spectrophotometer and a correction applied for the color of the sugar products themselves, the results were very erratic, and the method was found to be unsuitable for the analysis of colored factory products.

Poe and Edson[288] have used 2,4-dinitrophenol for the colorimetric determination of reducing sugars in food products and have obtained good checks with the Munson and Walker method.

Kolthoff's Dinitrosalicylic Acid Method for Determining Invert Sugar in Refined Sugars. Sumner[289] found that dinitrosalicylic acid is preferable to picric acid in the analysis of urine. It is also reduced to a deep red amino compound. Kolthoff[290] obtained good results with this reagent in estimating traces of invert sugar in refined sugars which do not contain enough coloring matter to interfere with the color of the reagent. Two grams of 3,5-dinitrosalicylic acid, $C_6H_2(NO_2)_2OHCOOH$, and 6 g. crystallized sodium carbonate are warmed with 70 ml. of water until they are completely dissolved. The solution is cooled, diluted to 100 ml., and filtered if necessary. Two grams of the sugar to be analyzed is dissolved in 10 ml. of warm water in a large test tube with a whirling motion. When the sugar is dissolved it is placed for 2 to 3 minutes in a water bath heated to 70° C. One milliliter of the dinitrosalicylic acid reagent is added, mixed with the sugar solution by rotating the tube, and finally 2 ml. of 4 *N* sodium hydroxide solution is added. This is also mixed with the rest of the solution, and the tube is placed for exactly 8 minutes in the water bath at 70° C. A blank is prepared in the same manner with 10 ml. of water, and the unknown is compared with it in a colorimeter. A column of the water blank 100 mm. high is matched by sugar solutions of varying invert sugar content as shown in the following table:

		HEIGHT OF COLUMN mm.
Pure sucrose		98
"	and 0.0025% invert	92
"	" 0.0050% "	80
"	" 0.0075% "	69
"	" 0.0100% "	58
"	" 0.0150% "	45

[287] *Z. Ver. deut. Zucker-Ind.*, 76, 273 (1926).
[288] *Ind. Eng. Chem., Anal. Ed.*, 4, 300 (1932).
[289] *J. Biol. Chem.*, 47, 4 (1921).
[290] *Arch. Suikerind.*, 30, 867 (1922).

These figures give a somewhat irregular curve. Better results would undoubtedly be obtained by using an appropriate color filter in order to obviate differences in tint in the two halves of the colorimetric field. It is advisable for the chemist to prepare his own standards with known invert sugar content and to compare these directly with the unknown. The addition of the reagents and all other operations must be carried out in exactly the same manner, as otherwise reproducible results cannot be obtained.

EXTRACTION OF SUGARS AND PREPARATION OF SOLUTIONS FOR CHEMICAL METHODS OF ANALYSIS

The methods and precautions previously given for the extraction of sugars and preparation of solutions for polarimetric examination hold also for the chemical methods of analysis.

Clarification of Sugar Products with Lead Salts

Precipitation of Reducing Sugars by Basic Lead Salts. Lead subacetate, or other basic salts of lead which are employed as clarifying agents in the polarimetric determination of sucrose, should never be used upon solutions in which reducing sugars are to be determined. The action of such compounds in causing a precipitation, or occlusion, of reducing sugars in the lead precipitate has already been mentioned. Bryan[291] found that basic lead salts, in the presence of magnesium sulfate and ammonium tartrate, precipitated in case of glucose from 3 per cent to 17 per cent, and in case of fructose from 8 per cent to 35 per cent, of the total amount of sugar in solution. Neutral lead acetate under the same conditions caused the precipitation of only 0.9 per cent of the total glucose and 0.0 per cent of the total fructose. (See Table LV, p. 322.)

In a series of independent experiments made by Bryan and Horne[292] upon raw cane sugar and cane molasses the results shown in Table CXX were obtained.

Clarification with lead subacetate caused a loss of about 10 per cent of the total reducing sugars present. The variable results, due to method of estimating copper, show a contamination of the cuprous oxide as explained on p. 771. The higher results by Allihn's method are caused by the greater reducing effect of the sucrose upon the more alkaline reagent.

[291] *Bull.* 116, U. S. Bur. Chem., p. 73.
[292] *Bull.* 116, U. S. Bur. Chem., pp. 72, 74.

TABLE CXX

INFLUENCE OF CLARIFICATION WITH LEAD SUBACETATE UPON DETERMINATION OF REDUCING SUGARS

	Clarifying Agent and Analyst	Allihn's Method			Munson and Walker's Method		
		Weighing as Cu_2O	Weighing as CuO	Titration of Cu by Low's Method	Weighing as Cu_2O	Weighing as CuO	Titration of Cu by Low's Method
		per cent	per cent	per cent	per cent	per cent	per cent
Cane Sugar	*No Clarifying Agent*						
	A. H. Bryan	6.45	6.22	5.88	6.29	5.98	5.83
	W. D. Horne	7.08	7.05	7.02	6.43	6.51	6.37
	Average	6.77	6.63	6.45	6.36	6.25	6.10
	Lead Subacetate Solution						
	A. H. Bryan	6.14	5.67	5.67	5.76	5.51	5.30
	W. D. Horne	6.61	6.51	6.51	6.19	6.01	5.99
	Average	6.38	6.09	6.09	5.98	5.76	5.65
Cane Molasses	*No Clarifying Agent*						
	A. H. Bryan	19.77	19.37	19.45	19.20	18.34	18.43
	W. D. Horne	20.60	20.06	19.97	20.00	19.43	19.44
	Average	20.19	19.72	19.71	19.60	18.89	18.94
	Lead Subacetate Solution						
	A. H. Bryan	17.51	16.47	16.29	17.27	16.26	15.97
	W. D. Horne	19.45	19.16	19.16	19.00	18.53	18.26
	Average	18.48	17.82	17.73	18.14	17.39	17.12

The results of Bryan and Horne have been fully confirmed by Deerr, Pellet, and others. It has also been shown that the reducing sugars are probably present in the precipitate as lead compounds. They cannot be removed by washing with water or by the usual deleading agents, but the compounds are broken up by treatment with acid.

Clarification with Neutral Lead Acetate. As has been stated above, neutral lead acetate does not precipitate any appreciable quantities of reducing sugars from impure products, and if the proper deleading agent is used, the recovery of reducing sugars is practically quantitative.[293] With products such as sugars, sirups, molasses, honey, and jellies, a minimum quantity of saturated lead acetate solution is added to the solution of the sugar product in a volumetric flask, the volume completed to the mark, and the solution filtered.

The problem of deleading is complicated by the fact, discovered by

[293] Saywell and Phillips, *Ind. Eng. Chem., Anal. Ed.*, 6, 116 (1934).

Eynon and Lane,[294] that it is necessary to remove not only the excess lead, but also the calcium usually present in impure sugar products, because calcium salts depress the reducing power of the reducing sugars. Sulfates and carbonates do not completely remove either the lead or the calcium, and they should therefore not be used as deleading agents.

Disodium phosphate completely removes the lead, but not all the calcium; sodium or potassium oxalate removes the calcium completely, but not all the lead. According to Eynon and Lane the small quantity of lead left after deleading with oxalate has no effect on the copper reduced, and they recommend the addition of a minimum of dry potassium oxalate to the filtrate from the lead precipitate. Cook and McAllep,[295] on the other hand, consider it best to remove both lead and calcium by adding a solution containing 7 g. disodium phosphate and 3 g. potassium oxalate in 100 ml. The required quantity of this solution is added to an aliquot of the filtrate from the lead precipitate, and the filtrate again made up to a definite volume. This method is used officially in the Hawaiian Islands. The United States Treasury Department prescribes clarification with neutral lead acetate, and deleading of the filtrate with dry potassium oxalate. Saywell and Phillips obtained the highest recovery of invert sugar by deleading with sodium oxalate instead of potassium oxalate, and ascribe this to the lower solubility of the sodium salt.

The question whether clarification with lead acetate is necessary in the case of cane products has not been settled satisfactorily. Cook and McAllep found that Hawaiian molasses contains reducing non-sugars which are removed by lead acetate. On the other hand, Eynon and Lane, also Meade,[296] could find no evidence of the presence of such substances. Meade even claims that the use of neutral lead acetate in any amount may lead to considerable errors in the determination of reducing sugars. He recommends filtration of the solution with the aid of a small quantity of dry kieselguhr, with the addition of sufficient dry potassium oxalate to remove the calcium.

However, in view of the positive evidence obtained by Cook and McAllep, it is safer to clarify with a minimum of neutral lead acetate and to delead either with dry sodium or potassium oxalate, or with Cook and McAllep's reagent mentioned above.

In the case of high-purity products which contain but little mineral matter or organic non-sugars, the use of lead acetate may be dispensed with, and a little dry kieselguhr may be added after completing the vol-

[294] *J. Soc. Chem. Ind.*, **42**, 143T (1923).
[295] *Hawaiian Planters' Record*, **32**, 142 (1928).
[296] "Spencer's Handbook for Cane-Sugar Manufacturers," 7th ed., p. 238, 1929.

ume, or a few milliliters of alumina cream before making up to the mark.

Douwes Dekker and Klokkers[297] have shown that the error caused by omitting clarification with neutral lead acetate and subsequent deleading does not exceed the experimental error in the reducing-sugar determination, in the analysis of white consumption sugars and even of raw sugars. The Java Sugar Experiment Station has therefore decided to abandon clarification for these products, but to continue its use for molasses sugars and other factory products.

As in the clarification of sugar products prior to polarization, the addition of lead acetate solution, and of deleading agents such as that of Cook and McAllep, causes a volume error the extent of which has not been ascertained.

Preparation of Sugar Solutions from Plant Substances

If the material to be analyzed contains much insoluble matter, as is the case with plant substances containing cellular tissue, the sugars must first be extracted by means of an appropriate solvent. Water has not been found satisfactory for the purpose, because of the action of enzymes upon sucrose, starch, and other higher saccharides. The employment of hot water is also often unreliable on account of the solution of hemicelluloses, starch, and gums.

Extraction of Sugars from Grains with Dilute Alcohol. Bryan, Given, and Straughn[298] made experiments upon the extraction of sugars from grains and similar products, using as solvents 50 per cent alcohol and 0.2 per cent sodium carbonate solution. Both these solvents inhibit the action of enzymes and were found to give concordant results upon certain classes of products. In many cases, however, the sodium carbonate extraction gave much higher amounts of reducing sugar after inversion — a result, perhaps, of the solvent action of the alkali upon pentosans and other hemicelluloses. Bryan, Given, and Straughn believe that extraction with 50 per cent alcohol, all points considered, is the most reliable method.

For the analysis of grains, cattle feeds, and similar materials, the method is carried out as follows, according to the directions of the Association of Official Agricultural Chemists:[299]

Place 10 g. of the material in a 250-ml. volumetric flask. If the substance has an acid reaction, add 1–3 g. of calcium carbonate to neutralize the acidity.

[297] *Arch. Suikerind.*, 41, II, 1089 (1933); Douwes Dekker and Goslings, *Arch. Suikerind.*, 42, II, 527 (1934).

[298] *Circular* 71, U. S. Bur. Chem.

[299] "Methods of Analysis, A. O. A. C.," 5th ed., pp. 358–359, 1940.

Add 125 ml. of 50 per cent alcohol by volume, mix thoroughly, and boil on a steam bath for 1 hour, using a small funnel in the neck of the flask to condense the vapor. Cool, and allow the mixture to stand several hours, preferably overnight. Make up to volume with neutral 95 per cent alcohol, mix thoroughly, and allow to settle. Pipette 200 ml. of the supernatant solution into a beaker and evaporate on a steam bath to a volume of 20–30 ml. Do not evaporate to dryness. A little alcohol in the residue does no harm. Transfer to a 100-ml. volumetric flask and rinse the beaker thoroughly with water, adding the rinsings to the contents of the flask. Add enough saturated neutral lead acetate solution (approximately 2 ml.) to produce a flocculent precipitate, shake thoroughly, and allow to stand 15 minutes. Dilute to the mark with water, mix thoroughly, and filter through a dry filter. Add sufficient anhydrous sodium carbonate or potassium oxalate to the filtrate to precipitate all the lead, again filter through a dry paper, and test the filtrate with a little anhydrous sodium carbonate or potassium oxalate to make sure that all the lead has been removed.

Twenty-five milliliters of the clear filtrate (equivalent to 2 g. of original material) is used for the determination of reducing sugars by Munson and Walker's or by Allihn's method.

For the determination of sucrose, introduce 50 ml. of the solution prepared as directed above into a 100-ml. volumetric flask, add a piece of litmus paper, neutralize with hydrochloric acid, add 5 ml. of hydrochloric acid, and allow the inversion to proceed at room temperature as directed on p. 414. When inversion is complete, transfer the solution to a beaker, neutralize with sodium carbonate, return the solution to the 100-ml. flask, dilute to the mark with water, filter if necessary, and determine reducing sugars in 50 ml. of the solution (representing 2 g. of the sample). Calculate the results as invert sugar. Subtract the percentage of reducing sugars before inversion from the percentage of total sugar after inversion, both calculated as invert sugar, and multiply the difference by 0.95 to obtain the percentage of sucrose present.

Because the insoluble material of grain or cattle food occupies some space in the flask as originally made up, it is necessary to correct for this volume. To obtain the true quantity of sugars present multiply all results by the factor 0.97, as results of a large number of determinations on various materials have shown the average volume of 10 g. of material to be 7.5 ml.

Extraction of Sugars from Plants in General. In the case of plants the Association of Official Agricultural Chemists prescribes stronger alcohol for the extraction of sugars, than in the case of grains and similar materials. The method is as follows:[300]

Thoroughly remove all foreign matter and rapidly grind or chop the material into fine pieces. Add the weighed sample to sufficient hot redistilled 95 per cent alcohol to which sufficient precipitated calcium carbonate has been added to neutralize the acidity, using sufficient alcohol so that the final concentra-

[300] *Op. cit.*, pp. 125 and 138.

tion, allowing for the water content of the sample, will be approximately 80 per cent. Heat close to the boiling point on a steam or water bath for 30 minutes, stirring frequently. The samples may be stored until needed for analysis.

In making the analysis pour the alcoholic solution through a filter paper or extraction thimble, catching the filtrate in a volumetric flask. Transfer the insoluble material to a beaker, cover with 80 per cent alcohol, warm on a steam bath for 1 hour, allow to cool, and again pour the alcoholic solution through the same filter. If the second filtrate is highly colored, repeat the extraction. Transfer the residue to the filter, allow to drain, and dry. Grind the residue so that all the particles will pass through a 1-mm. sieve, then transfer it to an extraction thimble and extract for 12 hours in a Soxhlet apparatus with 80 per cent alcohol. Dry the residue and save for the starch determination. Combine the alcoholic filtrates and make to volume at a definite temperature with 80 per cent alcohol.

Place an aliquot of the alcoholic extract in a beaker on the steam bath and drive off the alcohol. Avoid evaporation to dryness by adding water if necessary. When the odor of alcohol has disappeared from the sample, add about 100 ml. of distilled water and heat to 80° to soften gummy precipitates and break up insoluble masses. Cool to room temperature.

Transfer the solution to a volumetric flask and rinse the beaker thoroughly with water, adding the rinsings to the contents of the flask. Add enough saturated neutral lead acetate to produce a flocculent precipitate, shake thoroughly, and allow to stand 15 minutes. Test the supernatant liquid with a few drops of saturated lead acetate. If more precipitate forms, shake and allow to stand again; if no further precipitate forms, dilute to the mark with water, mix thoroughly, and filter through a dry filter. Add sufficient solid sodium oxalate to the filtrate to precipitate all the lead, and refilter through a dry paper. Test the filtrate for the presence of lead with a little solid sodium oxalate.

Determine the reducing sugars by the Munson and Walker or by the Quisumbing and Thomas method. The sucrose is determined in another aliquot of the solution, by the same reduction methods, after hydrolysis with hydrochloric acid, or better with invertase.

If only small amounts of reducing sugars are present the macro-methods do not give reliable results, and Hassid[301] recommends in this case the use of the ferricyanide-ceric sulfate micromethod (p. 876). The excess lead must be removed with disodium phosphate and not with oxalate, which would reduce the ceric sulfate. For the same reason all coloring matter must also be removed with a minimum of an efficient decolorizing carbon, such as Carboraffin.

Clarification with Mercuric Nitrate. Thomas and Dutcher[302] observed during an investigation on the determination of reducing sugars

[301] *Ind. Eng. Chem., Anal. Ed.*, 8, 138 (1936).
[302] *J. Am. Chem. Soc.*, 46, 1662 (1924).

in the leaves and spurs of the apple tree that lead acetate does not remove certain reducing non-sugars present in these materials. They recommend the use of Patein and Dufau's mercuric nitrate reagent (see below) diluted with an equal volume of water. A slight excess of this reagent is added to the plant extract after removal of the alcohol and dilution with water. The excess mercury is removed by carefully adding small quantities of dry sodium bicarbonate until the solution is just alkaline to litmus, but avoiding an excess. After filtration the last traces of mercury are removed by the addition of zinc dust and 1 drop of hydrochloric acid. A portion of the filtrate is tested for mercury with ammonium sulfide.

Preparation of Sugar Solutions from Animal Substances

Liquids of animal origin, such as blood, serum, urine, milk, secretions, and extracts, frequently contain large amounts of albuminoids and other nitrogenous substances which interfere with the determination of sugars by reduction methods, and they must be removed. It is especially important to remove reducing non-sugars, and this is complicated by the fact that the reducing effect of these non-sugars varies with the nature of the reagent used for the sugar determination. A clarifying agent which may give true sugar values with a given copper reagent for one biological material may not do so with another biological material, or with another copper reagent, or with a ferricyanide reagent, etc. If the sugar to be determined is fermentable, the safest course is to test the clarified solution for reducing non-sugars by making a second reduction experiment after fermentation with an appropriate yeast strain (see Chapter XV).

Clarification with Mercuric Nitrate. A clarifying agent which has been widely used for biological fluids is mercuric nitrate. It is prepared as follows, according to Patein and Dufau:[303] 220 g. of yellow mercuric oxide is gradually stirred into 160 ml. of concentrated nitric acid. The mixture is boiled and cooled, and then 60 ml. of 5 per cent sodium hydroxide solution is added. It is then diluted to 1 liter, filtered through asbestos, and kept in a dark bottle.

The liquid to be clarified is treated with the mercuric nitrate reagent until no more precipitate forms; the solution is then nearly neutralized with sodium hydroxide or better with sodium bicarbonate. A measured portion of the filtrate is freed from excess of mercury by precipitating with hydrogen sulfide; the solution is filtered, the hydrogen sulfide re-

[303] *J. pharm. chim.*, **15**, 221 (1902).

moved by a current of air, and the reducing sugars determined by any of the usual methods.

In the analysis of blood West, Scharles, and Peterson[304] proceed as follows. Five milliliters of blood is laked in 40 ml. of water in a 150-ml. Erlenmeyer flask. Slowly add from a burette, under constant shaking, 5 ml. of the Patein and Dufau reagent. Stopper the flask and shake vigorously. Add 3 g. precipitated barium carbonate and whirl the flask until most of the liberated carbon dioxide has escaped. Stopper the flask, shake vigorously, and then release the pressure by lifting the stopper. Repeat this until no further pressure develops on shaking. Filter, and test the filtrate with blue litmus paper, which should not be reddened. If it is, more barium carbonate is added and the process repeated. Now add 1 drop of concentrated sulfuric acid and 0.3 g. anhydrous sodium sulfate. Precipitate the mercury with hydrogen sulfide, filter, and remove the excess hydrogen sulfide by blowing air through the liquid. Add 1 drop of 10 per cent copper sulfate solution to take out the last trace of hydrogen sulfide. Filter, or centrifuge and filter the supernatant liquid. Five milliliters of the final filtrate, equal to 0.5 ml. of blood, is used for the sugar determination, the free acid being neutralized before the addition of the copper or other reagent.

Clarification with Mercuric Sulfate. Later, West, Scharles, and Peterson[305] introduced the use of mercuric sulfate instead of nitrate. The sulfate reagent is easily prepared by dissolving 30 g. of the sulfate in 10 per cent sulfuric acid to a total volume of 100 ml. For the clarification of blood, 5 ml. of the blood is laked in 50 ml. of water, and 5 ml. of the mercuric sulfate reagent is added. Further treatment is the same as described in the preceding paragraph, except that 9 to 10 g. of barium carbonate must be added instead of 3 g. Five milliliters of the final filtrate represents 0.417 ml. of the original blood.

Shaffer and Somogyi[306] have found this reagent serviceable for sugar determinations in urine, but it does not remove the reducing non-sugars completely, and the fermentable sugar should be determined by measuring the reducing value before and after treatment with yeast.

Clarification with Tungstic Acid. The use of tungstic acid is due to Folin and Wu.[307] In blood analysis, 1 volume of the blood is diluted with 7 volumes of water; 1 volume of a 10 per cent solution of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) is mixed with the diluted blood. Then 1 volume of $\frac{2}{3}$ *N* sulfuric acid is added; the flask is closed with a rubber

[304] *J. Biol. Chem.*, **82**, 137 (1929).
[305] *J. Biol. Chem.*, **82**, 137 (1929).
[306] *J. Biol. Chem.*, **100**, 695 (1933).
[307] *J. Biol. Chem.*, **38**, 81 (1919).

stopper and shaken vigorously. The filtrate is nearly neutral in reaction. Like the mercuric sulfate reagent, tungstic acid does not remove reducing non-sugars completely.

For microdeterminations of sugar in blood Folin and Svedberg recommend the following clarification procedure.[308] Place 10 g. anhydrous sodium sulfate and 15 ml. of 10 per cent sodium tungstate solution in a 500-ml. volumetric flask, shake until all is dissolved, and make to the mark. Keep this solution in a dark bottle. Transfer 4 ml. of the reagent to a 15-ml. centrifuge tube, add 0.1 ml. of blood, stir, and let stand for 15 minutes. Add 1 ml. of 0.08 *N* sulfuric acid, mix thoroughly without violent agitation, centrifuge for 15 minutes, and pipette off an aliquot for the sugar determination.

Clarification with Ferric Hydroxide. This method is due to Steiner, Urban, and West.[309] Twenty-one grams of ferric sulfate (Mallinckrodt's analytical reagent) is dissolved in water and diluted to 100 ml. Five milliliters of blood is laked in 50 ml. of water, and 5 ml. of the ferric sulfate reagent is mixed with it. Then 7 g. of precipitated barium carbonate is added, and the mixture is shaken in the stoppered flask, the pressure being released from time to time by lifting the stopper, until the solution is neutral to litmus paper. Five milliliters of the filtrate correspond to 0.417 ml. of the original blood. This reagent gives good results with serum and with urine, but it is advisable to make a test for reducing non-sugars with yeast. It may be used also for the clarification of milk, after dilution with water in the ratio of 1 to 20.

For microdeterminations of sugar in blood, 0.2 ml. of blood is used, laked in 7.6 ml. of water, and 0.2 ml. of ferric sulfate reagent is added in a 15-ml. centrifuge tube. Add 0.3 to 0.5 g. barium carbonate, shake vigorously, centrifuge, and filter. Five milliliters of the filtrate, equal to 0.125 ml. of blood, is used for the sugar determination.

Clarification with Zinc Hydroxide. This reagent, introduced by Somogyi.[310] removes not only protein and other interfering substances from blood, but also non-sugars having a reducing effect on the Shaffer and Somogyi copper reagent (see p. 846). A 10 per cent solution of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) is prepared, and its concentration adjusted so that 10 ml. of it, diluted with about 50 ml. of water, requires 10.8 to 11.2 ml. of 0.5 *N* sodium hydroxide solution for neutralization, with phenolphthalein as indicator. One volume of blood is diluted with 7 volumes of water, and 1 volume of the zinc sulfate solution is added and thoroughly mixed. Then 1 volume of 0.5 *N* sodium hydroxide is added

[308] *J. Biol. Chem.*, **88**, 85 (1930).
[309] *J. Biol. Chem.*, **98**, 289 (1932).
[310] *J. Biol. Chem.*, **86**, 655 (1930).

with constant agitation; the flask is stoppered and well shaken. Five milliliters of the filtrate, representing 0.5 ml. of blood, is used for the sugar determination by the method of Shaffer and Somogyi.

For microdeterminations, a 1.8 per cent solution of zinc sulfate, 10 ml. of which neutralizes 12 to 12.2 ml. of 0.1 *N* sodium hydroxide, is employed. Either 0.1 ml. of blood plus 5.9 ml. of water, or 0.2 ml. of blood plus 5.8 ml. of water, is used. In either case 1 ml. of the zinc sulfate solution and 1 ml. of 0.1 *N* sodium hydroxide are added. Five milliliters of the filtrate corresponds to 0.0625 or 0.125 ml., respectively, of the original blood.

Clarification with Dry Basic Zinc Acetate. Instead of producing a precipitate of zinc hydroxide in the solution to be analyzed, Letonoff[311] prepares the clarifying agent in the form of a dry powder and adds it to the solution. A solution of 39.6 g. sodium hydroxide in 400 ml. of water is added with stirring to 120 g. zinc acetate, $Zn(C_2H_3O_2)_2 \cdot 2H_2O$, dissolved in 1600 ml. water. The precipitate is filtrated off by suction and washed with water until the filtrate is neutral to phenol red indicator. It is then dried at room temperature on filter paper or a porous plate, ground, and sifted. The material which passes an 80-mesh sieve and is retained on 100 mesh is used. About 1 g. of it is added for 1 ml. of blood diluted 1 : 10; for plasma or serum 3 g. per ml. The filtrate contains a trace of zinc which does not interfere with the sugar determination, but may be removed if desired, with a little sodium carbonate. The reagent eliminates reducing non-sugars, like Somogyi's zinc reagent, and has the further advantage that no salts are added to the solution, and that no error is introduced by the volume occupied by the precipitate.

Clarification of Milk. For the clarification of milk, the use of copper sulfate and potassium hydroxide will be found convenient. The following is the official method of the Association of Agricultural Chemists:[312]

> Dilute 25 g. of the milk with 400 ml. of water and add 10 ml. of a solution of copper sulfate of the strength given for Soxhlet's modification of Fehling's solution. Add about 7.5 ml. of a solution of potassium hydroxide of such strength that 1 volume of it is just sufficient to completely precipitate the copper as hydroxide from 1 volume of the solution of copper sulfate. Instead of a solution of potassium hydroxide of this strength, 8.8 ml. of a half-normal solution of sodium hydroxide may be used. After the addition of the alkali solution the mixture must still have an acid reaction and contain copper in solution. Fill the flask to the 500-ml. mark, mix, and filter through a dry filter. Determine the lactose by any of the usual methods.

[311] *J. Biol. Chem.*, 106, 693 (1934).

[312] "Methods of Analysis, A. O. A. C.," 5th ed., p. 272, 1940.

Since lime salts diminish the reducing power of sugars, more accurate results are obtained if the calcium is removed from the clarified solution by the addition of dry potassium oxalate, followed by a second filtration. A correction should be applied for the volume of the precipitate formed during clarification, by multiplying the lactose found by the factor 0.9875.

Clarification Procedure for Meat and Meat Products. For the determination of sugar in meat and meat products, the Association of Official Agricultural Chemists prescribes clarification with a solution of 100 g. of phosphotungstic acid in 100 ml. of water.[313]

Weigh 100 g. of the finely ground sample into a 600-ml. beaker, add 200 ml. of water, heat to boiling, and boil gently for 5 minutes. Stir the contents of the beaker frequently during this and subsequent extractions to prevent bumping. (When several samples are extracted at the same time a mechanical stirring device is practically a necessity.) Remove the beaker from the flame, allow the insoluble matter to settle, and decant the clear liquid on an asbestos mat in a 4-inch funnel. Filter with the aid of suction. Add 150 ml. of hot water to the residue in the beaker, boil gently for 5 minutes, let settle, and decant the clear liquid as directed previously. Repeat the operation, finally transfer the contents of the beaker to the funnel, wash with 150–200 ml. of hot water, and press the meat residue as dry as possible. Transfer the contents of the filter flask to an evaporating dish and evaporate on a steam bath to a volume of about 25 ml. but not to dryness. Transfer the extract to a 100-ml. volumetric flask, taking care that the volume of liquid does not exceed 60 ml. Then add 25–35 ml. of phosphotungstic acid solution, shake vigorously, let stand a few minutes for gas bubbles to rise to the surface, make to volume, shake, and either filter or centrifuge. (The use of a centrifuge is to be preferred, because a larger volume of liquid is obtained.) Test a portion of the filtrate with dry phosphotungstic acid for complete precipitation. If an appreciable precipitate forms, take an aliquot of the filtrate, add 5–10 ml. of the phosphotungstic acid solution, make to volume, filter, and test the filtrate for complete precipitation. The filtrate should also show not more than a slight reaction for creatinin when tested by adding to 5 ml. a few drops of a saturated aqueous solution of picric acid and making the mixture alkaline with a few drops of 10 per cent sodium hydroxide.

To determine the total sugar after inversion, transfer 50 ml. of the clarified extract to a 100-ml. volumetric flask, add 5 ml. of hydrochloric acid and invert the solution with the usual precautions. Cool the solution, neutralize to litmus, cool, make to volume, and filter. To the filtrate add sufficient dry powdered potassium chloride to precipitate the excess of phosphotungstic acid, filter, test the filtrate for complete precipitation, and determine the total reducing sugar as glucose.

If an abnormal reduction is obtained when the clarified meat extract is

[313] " Methods of Analysis, A. O. A. C.," 5th ed., pp. 379–380, 1940.

boiled with Fehling's solution, i.e., if the solution turns yellow, brown, green, or muddy in appearance instead of reddish blue, discard the determination, since incomplete precipitation of the nitrogenous compounds, due to the use of insufficient phosphotungstic acid, is indicated.

In the case of meat extracts,[314] 20 g. of sample is heated on the steam bath with about 200 ml. of water until all soluble substances have gone into solution; the aqueous extract is then treated as described in the preceding paragraphs.

In determining reducing sugars in substances of animal origin, the precipitate of cuprous oxide is often badly contaminated with mineral and organic impurities, so that the reduced copper should be determined directly and not by weight of suboxide, oxide, or oxide reduced to metal.

Pretreatment with a Combination of Clarifying Agents. At times the complexity of a material to be analyzed makes it necessary to use several preparatory treatments and clarifying agents in succession. As an example the determination of milk products in mixed feeds is cited. Mixed feeds may contain various ground grains or other seeds, the by-products of the grain-milling and starch-products industries, molasses, oil meals, fish meal, meat by-products, oils, and mineral substances. In the method devised for lactose determination in such mixed feeds by Magraw and Sievert,[315] the material is first extracted with ether, and then treated with diastase, invertase, and melibiase to convert starch, sucrose, and raffinose into fermentable sugars. The resulting mixture is fermented with baker's yeast. After fermentation the solution is clarified with neutral lead acetate, and the filtrate with mercuric chloride and phosphotungstic acid. The excess lead and mercury are precipitated with hydrogen sulfide, and after removal of the latter the copper-reducing power is determined and calculated as lactose. If it is known what dairy product has been used in the compounding of the feed, whether dry skim milk, dried buttermilk, or dried whey, its percentage can be computed from the lactose found.

In each special case the chemist must choose the clarifying agents in such a way that all interfering substances are removed, without affecting the reducing sugars to be determined.

Concentration of Sugar Solutions

In working with very dilute solutions, such as contain only a few hundredths of a per cent of sugar, it is necessary either to use micro-methods, or to concentrate the liquid to one-half, one-fifth, or one-tenth

[314] "Methods of Analysis, A. O. A. C.," 5th ed., p. 386, 1940.
[315] *Ind. Eng. Chem., Anal. Ed.*, 7, 106 (1935).

the original volume before a satisfactory determination of the copper-reducing power can be made. It is exceedingly important in evaporating such solutions that the liquid be kept exactly neutral; otherwise changes may result in the composition of the sugars. Traces of free acid may become sufficiently concentrated towards the end of evaporation to hydrolyze higher saccharides, and traces of free alkali may modify or destroy reducing sugars.

The evaporation of solutions containing reducing sugars must be conducted in vessels which do not give up soluble alkali; the concentration of sugar solutions in glass vessels, unless of perfectly resistant non-soluble quality, is for this reason to be avoided. Flasks and basins of tinned copper are very suitable for concentrating sugar solutions, there being no change in reducing power after diluting and evaporating to the original volume.

If the solution to be concentrated is slightly acid an excess of finely powdered calcium carbonate (alkali free) will prevent the hydrolysis of higher saccharides. If the solution is alkaline, dilute acetic acid is first added to faint acidity, and then an excess of calcium carbonate. When the evaporation is completed, the residue of insoluble matter is removed by filtration.

Evaporation under reduced pressure also minimizes the danger of hydrolysis or attack by alkali.

CHAPTER XV

SPECIAL QUANTITATIVE METHODS

The methods based on the effect of reducing sugars upon alkaline solutions of copper or mercury salts and of some other compounds are, with a few exceptions, applicable to all reducing sugars and do not permit the selective determination of particular groups of them. For such purposes more special processes of analysis must be adopted. The present chapter will describe a number of the best known of such special quantitative methods.

DETERMINATION OF ALDOSES BY MEANS OF HYPOIODITE

Romijn[1] discovered that iodine in weakly alkaline solution oxidizes aldoses to the corresponding monobasic acids. Under controlled conditions the oxidation of the aldoses is quantitative, while ketoses and non-reducing sugars are only slightly attacked.

The reaction proceeds according to the following equation:[2]

$$RCOH + I_2 + 3\,NaOH \longrightarrow RCOONa + 2\,NaI + 2\,H_2O$$

The iodine and alkali first form hypoiodite and iodide:

$$I_2 + 2\,NaOH \longrightarrow NaIO + NaI + H_2O$$

Depending on the concentration, time, and temperature, a greater or smaller part of the hypoiodite is converted into iodate and iodide:

$$3\,NaIO \longrightarrow NaIO_3 + 2\,NaI$$

When the oxidation of the aldose is complete, the solution is acidified, and the excess iodine is liberated according to the equations

$$NaIO + NaI + 2\,HCl \longrightarrow I_2 + 2\,NaCl + H_2O$$

and

$$NaIO_3 + 5\,NaI + 6\,HCl \longrightarrow 3\,I_2 + 6\,NaCl + 3\,H_2O$$

In either case one molecule of iodine (I_2) is obtained for each molecule of hypoiodite. The liberated iodine is titrated with thiosulfate, and the difference between the total iodine added, and that found at

[1] *Z. anal. Chem.*, 36, 349 (1897).

[2] See Kline and Acree, *Bur. Standards J. Research*, 5, 1063 (1930).

the end of the experiment, gives the amount of iodine corresponding stoichiometrically to that of the aldose used. This is a great advantage over copper-reduction methods, where empirical tables must be employed.

In strongly alkaline solutions the oxidation proceeds beyond the aldonic acid stage, and for this reason Romijn used borax to furnish the weakly alkaline medium. Complete oxidation required about 18 hours at 25° C. The method has since been studied by many investigators, and only a few of the more important procedures proposed will be described here.

Method of Willstätter and Schudel.[3] These authors found that the time necessary for complete oxidation can be greatly reduced. A measured quantity of a solution of glucose or other aldose is treated with about twice the amount of *N*/10 iodine solution necessary for complete oxidation. A quantity of *N*/10 sodium hydroxide solution, equivalent to about one and one-half times that of the iodine used, is added drop by drop, and the mixture is allowed to stand for about 12 to 15 minutes, or even 20 minutes. The solution is now acidified with dilute sulfuric or hydrochloric acid, and the liberated iodine is titrated with *N*/10 thiosulfate. One milliliter of *N*/10 iodine used for oxidation is equivalent to 7.004 mg. of an aldopentose, 9.005 mg. of an aldohexose, and 17.11 mg. of a disaccharide aldose as maltose or lactose.

Example. To 10 ml. of a solution containing 100 mg. of glucose, and requiring 100 ÷ 9.005 or 11.11 ml. *N*/10 iodine for oxidation, are added 20 ml. of the iodine solution, and gradually with stirring 30 ml. of *N*/10 sodium hydroxide. At the end of the reaction 8.95 ml. of *N*/10 thiosulfate is required. The iodine used for the oxidation equals therefore 20 − 8.95, or 11.05 ml. Found 11.05 × 9.005, or 99.5 mg. glucose.

Higher concentrations or larger volumes of the reagents may also be used, as long as the proportions remain the same. For very small quantities of sugar it is preferable to use *N*/50 or *N*/100 solutions.

Kolthoff[4] recommends a somewhat larger excess of iodine, two and a half times that required, without a change in the alkali added. In the above example 25 ml. of *N*/10 iodine, and 30 ml. of *N*/10 alkali would be employed. Oxidation is complete in 3 to 5 minutes for glucose, galactose, arabinose, or maltose; but lactose requires about 10 minutes' standing. Kolthoff found that under the above conditions each gram of fructose absorbs 1.2 ml. of *N*/10 iodine, and can thus be corrected for. Sucrose has a smaller effect; it takes up from 0.35 to

[3] *Ber.*, **51**, 780 (1918).

[4] *Z. Untersuch. Nahr. u. Genussm.*, **45**, 131 (1923).

0.6 ml. *N*/10 iodine per gram, depending on the concentration and the time. Alcohol, glycerol, mannitol, formic acid, lactic acid, dextrin, amino acids, and many other substances also take up iodine, and separately determined corrections must be applied for all of them when present. For this reason the method cannot be applied directly to impure sugar products of unknown composition.

Method of Auerbach and Bodländer.[5] In order to reduce the error caused by the presence of fructose, as for instance in the analysis of honeys, these authors employed a buffer solution of *p*H 10.1–10.2 instead of free alkali. Twenty-five milliliters of a solution containing 0.2 g. of honey is mixed with from one and a half to two times the quantity of *N*/10 iodine solution required for the complete oxidation of the glucose. Then a mixture of 50 ml. each of 0.2 *M* sodium carbonate and sodium bicarbonate solution is added, and the solution allowed to stand in the dark for 1½ to 2 hours. It is then acidified with 12 ml. of 25 per cent sulfuric acid, and the excess iodine titrated with *N*/10 thiosulfate. A blank titration is carried out in the same manner with 25 ml. of water instead of sugar solution, and the titer after oxidation of the glucose is deducted from the titer of the blank. Each milliliter of the difference indicates 9.005 mg. glucose. Under these conditions the error caused by 100 mg. of fructose amounts to only 1 mg. of glucose, and this may be deducted as a correction. The iodine used by 100 mg. of sucrose is so small as to be negligible.

Douwes Dekker[6] and also Lothrop and Holmes[7] tried this method with pure sugars and did not obtain complete oxidation in the time specified by Auerbach and Bodländer. Moreover, duplicate analyses did not check very well. The long time required for the determination is a further objection in routine analysis.

Other investigators who have examined the hypoiodite method with either sodium hydroxide or with buffer salts, as carbonate mixtures and phosphates, have not been able to obtain concordant results. It has been found that the course of the reaction is greatly affected not only by the relative proportions between sugar, iodine, and alkali, but also by the rapidity with which the alkali is added, and the time and temperature employed. If the alkali is added too rapidly, a large part of the hypoiodite is converted into iodate, and the iodine may become exhausted before the sugar has been completely oxidized. Too low results are obtained if the alkali is not sufficient, or the time allowed too

[5] *Z. angew. Chem.*, 36, 602 (1923); *Z. Untersuch. Nahr. u. Genussm.*, 47, 233 (1925).

[6] *Arch. Suikerind.*, 36, III, 699 (1928).

[7] *Ind. Eng. Chem., Anal. Ed.*, 3, 334 (1931).

short, or the temperature too low. If too large an excess of iodine and alkali are used, the oxidation is liable to go beyond the aldonic acid stage, and this may also happen if the time is too long or the temperature too high.

Method of Kline and Acree.[8] These authors have shown that the errors just mentioned may be avoided by using a well-defined excess of both iodine and alkali, and by adding these reagents alternately in several portions. At the completion of the reaction the solution is acidified with a measured quantity of acid, and after the iodine has been titrated with thiosulfate, the excess acid is titrated back with standard alkali. This makes it possible to check the result of the iodine titration by also measuring the alkali used in the reaction, according to the equation given on p. 895 and showing that 3 moles of alkali correspond to 1 of sugar.

The procedure employed by Kline and Acree is described as follows. Take an aliquot of the sugar solution or a weighed amount of the solid substance, which will react with approximately 20 ml. of *N*/10 iodine. If the solution is not neutral, add *N*/10 sodium hydroxide or *N*/10 hydrochloric acid, using phenolphthalein as indicator. Only 1 drop of the latter should be used, because the alcohol introduces a potential source for loss of iodine. To avoid this error, a water solution of phenol red or thymol blue may be used in this titration.[9] Add 5 ml. of *N*/10 iodine from a burette; then, with vigorous stirring, add drop by drop from a burette 7.5 ml. of *N*/10 sodium hydroxide. Repeat this process until 22 ml. of iodine and 35 ml. of alkali have been run in. This operation will take about 5 to 6 minutes. Allow a 2-minute interval for the completion of the oxidation. Acidify with *N*/10 (or *N*/5) hydrochloric acid to free the iodine from any sodium iodate present and titrate the liberated iodine with *N*/10 thiosulfate. Starch indicator may be used. Add 2 to 3 drops of phenolphthalein solution and titrate the excess acid with *N*/10 sodium hydroxide.

If the iodine liberated by acidification requires more than 2 to 3 ml. of thiosulfate, too much iodine has been added, resulting in overoxidation. If a very accurate determination is desired, the experiment is repeated, and the amount of the thiosulfate titration less 2 ml. is deducted from the amount of iodine to be added to the unknown. On the other hand, if the thiosulfate titration after acidification is less than 1.5 ml., a sufficient amount of iodine has not been added. In such a case it is necessary to repeat the experiment adding more iodine and alkali.

[8] *Bur. Standards J. Research,* **5**, 1063 (1930).

[9] According to Mallen (*Analyst,* **57**, 244) phenolphthalein itself absorbs iodine, while methyl orange may be safely used.

When the solution is acidified, a small amount of the aldonic acid forms the corresponding lactone, and this causes the phenolphthalein indicator to fade during the final neutralization with alkali. If the alkali is added slowly, however, the pink color persists for about a minute when the end point is reached. Fading may be caused also by the carbon dioxide in the air, and if this is suspected it is better to carry out the entire reaction in a stoppered flask.

The number of milliliters of $N/10$ iodine minus the number of milliliters of $N/10$ sodium thiosulfate, and the number of milliliters total $N/10$ sodium hydroxide minus the number of milliliters of $N/10$ hydrochloric acid used give the quantities of $N/10$ iodine and $N/10$ sodium hydroxide required for the oxidation of the sugar. One millimole of the sugar (0.150 g. aldopentose, 0.180 g. aldohexose, 0.342 g. disaccharide aldose) requires for oxidation 20 ml. of $N/10$ iodine and 30 ml. $N/10$ sodium hydroxide.

Example. Fifty milliliters of solution containing 0.1529 g. xylose taken for analysis; 20.35 ml. $N/10$ iodine and 30.57 ml. $N/10$ alkali were consumed in oxidizing the xylose. Then

$$\text{Per cent xylose} = \frac{0.1500}{0.1529} \times \frac{20.35}{20.00} \times 100 = 99.81 \quad \text{(from iodine)}$$

$$\text{Per cent xylose} = \frac{0.1500}{0.1529} \times \frac{30.57}{30.00} \times 100 = 99.96 \quad \text{(from alkali)}$$

Glucose, galactose, lactose, and xylose could be determined satisfactorily by this method. Ketoses and non-reducing sugars are not oxidized sufficiently to interfere with the precision of the method unless iodine and alkali are used in excess of the amounts stated. Xylans and galactans also have no effect. However, phenolic compounds, as lignins, disturb the 2 : 3 ratio for iodine and alkali.

According to Miller,[10] the procedure of Kline and Acree gives slightly low results with lactose hydrate, 98.6 per cent of the theoretical, in the oxidation time of 8 minutes, but the reaction is complete in 15 minutes. Mixtures of equal parts of glucose and lactose require even more time for complete oxidation, while the addition of sucrose to the mixture speeds up the reaction time. Care must therefore be taken in interpreting the results of analysis by the hypoiodite method, and check analyses should be made with mixtures of pure sugars in about the same proportions as present in the sample.

[10] *Ind. Eng. Chem., Anal. Ed.*, 9, 37 (1937). See also Myrbäck and Örtenblad, *Svensk Kem. Tids.*, 50, 72 (1938); 51, 7 (1939).

Method of Lothrop and Holmes for Determining Glucose in Honey. A procedure which is somewhat simpler than that of Kline and Acree, but which nevertheless gives satisfactory results in the analysis of honey, has been described by Lothrop and Holmes.[11] It is similar to the method of Willstätter and Schudel. Transfer 20 ml. of a solution containing 0.2 g. of honey to a 250-ml. Erlenmeyer flask, and add 40 ml. of 0.05 *N* iodine solution. Run in 25 ml. of 0.1 *N* sodium hydroxide, stopper, and let stand for 10 minutes at 20° C. Acidify with 5 ml. of 2 *N* sulfuric acid and titrate at once with 0.05 *N* thiosulfate, using starch as an indicator. The weight of glucose in grams (not corrected for reduction of iodine by fructose) is found by multiplying the milliliters of reduced 0.05 *N* iodine by 0.004502.

Under these experimental conditions the fructose is slightly oxidized by the hypoiodite, owing to the Lobry de Bruyn and van Ekenstein rearrangement into glucose and mannose. A correction for this is applied in the final calculation. Sucrose is oxidized very slowly, and no correction need be applied for the small quantities present in genuine honeys.

The total reducing sugars are also determined in the honey by the Munson and Walker method; the approximate percentage of fructose is found by deducting the uncorrected glucose from the total reducing sugars calculated as glucose, and dividing by the reducing ratio 0.925. The true glucose is then calculated by deducting from the uncorrected glucose 0.012 per cent of the approximate fructose. Finally the true fructose is found by deducting the true glucose from the total reducing sugars calculated as glucose and dividing by 0.925. The sum of true glucose and true fructose gives the true total reducing sugars.

Analyses of a large number of American honeys gave results closely agreeing with those of Browne,[12] who used the low- and high-temperature polarization method for determining fructose.

Mannose is oxidized much more slowly than glucose, and the alkali must be added very gradually to insure complete oxidation.

A rapid semi-micromethod for determining maltose by oxidation with hypoiodite has been described by Caldwell, Doebbeling, and Manian.[13]

Method of Shapiro and Proferansova for Determining Maltose in the Presence of Sucrose, Glucose, and Fructose.[14] In this method the sucrose is first inverted. Hypoiodite treatment oxidizes the maltose

[11] *Ind. Eng. Chem., Anal. Ed.*, 3, 334 (1931).
[12] *Bull.* 110, U. S. Bur. Chem., p. 38.
[13] *Ind. Eng. Chem., Anal. Ed.*, 8, 181 (1936).
[14] *Z. Ver. deut. Zucker-Ind.*, 85, 196 (1935).

to maltobionic acid and the glucose to gluconic acid. The fructose is destroyed by heating with alkali, leaving the aldonic acids unaffected. The maltobionic acid is then hydrolyzed by heating with dilute acid, and the reducing power determined. The details of the procedure are as follows. Twenty milliliters of the solution, which after inversion of the sucrose should contain not more than 150 mg. aldoses (2 maltose being taken to be equivalent to 1 glucose), are acidified with 1 ml. concentrated hydrochloric acid, and the sucrose is inverted by heating for 5 minutes to 68–70° C. The solution is cooled and neutralized against phenolphthalein with *N* sodium hydroxide. The maltose and glucose are oxidized by adding 3.5 ml. of *N* iodine solution, then, drop by drop, 4.2 ml. of *N* sodium hydroxide, shaking, and allowing the well-stoppered flask to stand for 10 minutes. The solution is acidified again with about 4 ml. of *N* sulfuric acid, and the excess iodine is removed by carefully adding 10 per cent sodium sulfite solution until the solution has become light yellow, and finally with 1 per cent sodium sulfite solution. It is then neutralized with *N* sodium hydroxide, methyl orange being used as indicator, and evaporated in a flask of resistance glass on the water bath to a volume of 20 to 30 ml. Enough sodium hydroxide is added to give a concentration of 5 per cent, and the solution is heated for 2 hours longer in the water bath under reflux. The solution becomes yellow to brown, and a flocculent precipitate forms. The mixture is cooled to room temperature and slightly acidified with dilute hydrochloric acid (1 : 1), being cooled under running tap water. It is then transferred to a 100-ml. flask and made to the mark. A pinch of purified animal char is added, well mixed with the solution, and the mixture filtered.

Twenty milliliters of the filtrate is neutralized with sodium hydroxide, and the reducing power is determined by the method of Bertrand. The apparent glucose found is called *A*. Another 20-ml. portion of the filtrate is also neutralized with sodium hydroxide, and enough concentrated hydrochloric acid is added to give a concentration of 3 per cent. The solution is heated for 1½ hours in a boiling-water bath under reflux, cooled, neutralized with sodium hydroxide, and the glucose is determined as before (*B*). The maltose hydrate originally present is then calculated by multiplying the difference $B - A$ by 2. With mixtures of pure sugars the error in the maltose found is ± 2.5 per cent.

Hinton and Macara[15] have proposed the use of a mixture of chloramine T (see p. 425) and potassium iodide in the presence of alkali for the oxidation of aldoses, but this procedure requires more time than the usual hypoiodite method.

[15] *Analyst*, **52**, 668 (1927).

SPECIAL METHODS FOR DETERMINING FRUCTOSE

Determination of Fructose after Hypoiodite Treatment

Fructose can be determined selectively by first destroying any aldoses present with hypoiodite, and then measuring the remaining reducing power.

Kolthoff-Kruisheer Method for Determining Fructose. Kolthoff[16] briefly outlined a procedure based on this principle, and Kruisheer,[17] recognizing the advantages of the method for practical sugar analysis, worked it out in greater detail. When aldoses are determined selectively by the hypoiodite method there is always the possibility that other substances may be present which are also oxidized by hypoiodite. But the only reducing sugars remaining after the treatment must be ketoses, and of these fructose is the only one to be considered in the usual practice of sugar analysis. Kruisheer's method is carried out as follows:

Dissolve a suitable quantity of the product, representing from 1.75 to 3.5 g. total dry substance, in water in a 100-ml. volumetric flask, neutralize the solution, and complete the volume. Pipette 25 ml. of this solution into another 100-ml. flask, add 25 ml. of water, and then 5 ml. of 4 *N* sodium hydroxide. Run in at once 16 ml. of iodine solution (13 g. iodine and 15 g. potassium iodide in 100 ml.), or enough to impart a distinctly brown color to the solution. Let stand for 5 to 7 minutes, and add 3 ml. of 4 *N* sulfuric acid. Remove the excess iodine, first with 20 per cent sodium sulfite solution, and then carefully with 2 per cent sodium sulfite solution, until the liquid is only slightly colored with iodine. Add 4 drops of a 2 per cent starch solution, and continue running in the sodium sulfite solution slowly until the blue color is discharged. Add 4 *N* sodium hydroxide, until the solution is just slightly acid, using methyl orange as indicator. Complete the volume to 100 ml., and determine the fructose in an aliquot by the modified Luff-Schoorl method (p. 832). Sodium thiosulfate must not be used to remove the excess iodine because the tetrathionate formed reduces alkaline copper solutions.

Under these conditions fructose was recovered to the extent of 98.3 per cent. Glucose, sucrose, and lactose showed no residual reducing effect, and commercial glucose or corn sugar only a trace. In genuine honeys a slightly higher ratio of fructose to glucose was found than by the method of Auerbach and Bodländer (p. 897), but the opposite re-

[16] *Z. Untersuch. Nahr. u. Genussm.*, **45**, 146 (1923).
[17] *Z. Untersuch. Lebensm.*, **58**, 261 (1929).

sult was obtained with artificial honeys containing starch conversion products.

Klasing's Method for Determining Fructose.[18] In order to minimize the oxidizing effect on the fructose, Klasing used, instead of sodium hydroxide, a buffer solution prepared by dissolving 25 g. anhydrous sodium carbonate in about 600 ml. of water in a liter flask, gradually adding with shaking 95 g. of finely powdered sodium bicarbonate, and making up to the mark. Twenty milliliters of sugar solution, containing less than 400 mg. fructose and 100 to 150 mg. glucose, is mixed with 15 ml. of 0.3 *N* iodine solution, 40 ml. of the buffer solution is added, and the flask is stoppered and set aside in the dark at about 28° C. for 1 hour. The solution is acidified with 10 ml. dilute sulfuric acid (1 : 5), the greater part of the excess iodine removed with 20 per cent sodium sulfite solution, and the remainder by means of 1 per cent sulfite solution. Dilute sodium hydroxide is added until the reaction is neutral to methyl orange. The volume is completed to 100 ml., and the fructose determined in an aliquot.

With mixtures of glucose and fructose the results were found from 0.67 per cent too low to 1.7 per cent too high. Large quantities of sucrose, usually present in cane products, cause a decided plus error, for two reasons. The sucrose is oxidized to some extent by the iodine, and the oxidation product reduces alkaline copper solution. Second, the acid added after the iodine treatment inverts a part of the sucrose, and the invert sugar formed increases the fructose result. Klasing recommends therefore that 30 per cent acetic acid be used instead of the sulfuric acid, and that a blank determination be run with the same quantity of sucrose as is present in the solution analyzed. The result of the blank is deducted from the fructose figure found. The fructose found by copper reduction must be further corrected for the reducing effect of the sucrose. All the corrections for the errors due to the sucrose may be avoided by first inverting all the sucrose, determining the total fructose, and deducting from the result the fructose corresponding to the sucrose present (52.63 per cent of the sucrose), as found by double polarization.

Estimation of Fructose, Following Destruction by Acid Treatment

Sieben's Method. Sieben[19] in 1884 proposed a method for determining fructose which is based upon the destruction of this sugar when heated with dilute hydrochloric acid. The method was designed for

[18] *Arch. Suikerind.*, 38, I, 339; III, 1109 (1930).
[19] *Z. Ver. deut. Zucker-Ind.*, 34, 837, 865 (1884).

estimating fructose in honey, sirups, and other products which contain glucose. Glucose, like other aldoses, is much less susceptible to the destructive action of acids, so that the difference in the reducing power of a solution before and after treatment by Sieben's process was taken as the equivalent of the fructose present.

Later investigators found,[20] however, that under the conditions specified by Sieben the fructose is not completely destroyed, while the glucose is partly attacked. The extent of these effects varies with the ratio between the two sugars present. Lucius[21] tried to overcome these difficulties by changing the strength of acid and the time of heating, and by estimating the remaining glucose polarimetrically. But this and other attempts to modify and improve the method so as to overcome the objections have not been wholly successful.[22]

Fiehe's Hydroxymethylfurfural Method. Fiehe discovered[23] that when 25 ml. of a solution containing 1 per cent of fructose or 2 per cent of sucrose is heated with 10 ml. of 5 *N* hydrochloric acid for 30 minutes in a boiling-water bath, the hydroxymethylfurfural formed can be determined as the phloroglucide, as described on p. 923, while under the same conditions no phloroglucide is obtained from glucose, maltose, lactose, starch, or even arabinose. One milligram of the phloroglucide is equivalent to 2.135 mg. fructose or 4.065 mg. sucrose. If less than 11 mg. phloroglucide is obtained, the experiment must be repeated with a larger quantity of the product. In mixtures containing fructose and sucrose, the sucrose must be determined in a separate portion by double polarization, and the corresponding amount of fructose deducted from the result obtained, in order to find the fructose originally present.

DETERMINATION OF PENTOSES AND PENTOSANS

Theory of Method. The methods for determining pentoses and pentosans are due to the researches of Tollens[24] and his school; they all depend upon the conversion of the pentose sugars into furfural by distilling with hydrochloric acid, according to the principles described on p. 706. The amount of furfural which distils over is determined and

[20] Herzfeld, *Z. Ver. deut. Zucker-Ind.*, 35, 967 (1885); Wiechmann's "Sugar Analysis," p. 91, 1914; Dammüller, *Z. Ver. deut. Zucker-Ind.*, 38, 751 (1888).

[21] *Z. Untersuch. Nahr. u. Genussm.*, 38, 177 (1919); 46, 94 (1923); 51, 351 (1926).

[22] Fiehe and Kordatzki, *Z. Untersuch. Lebensm.*, 62, 516 (1931); Klasing, *Arch. Suikerind.*, 38, I, 339 (1930).

[23] *Z. Untersuch. Lebensm.*, 63, 288 (1932).

[24] For a review of the subject see papers by Tollens with bibliography in Abderhalden's "Arbeitsmethoden," 1909, II, 130.

calculated to pentoses. The yield of furfural does not correspond perfectly to the equation

$$\underset{\text{100 parts pentose}}{C_5H_{10}O_5} \longrightarrow \underset{\text{64 parts furfural}}{C_5H_4O_2} + 3\,H_2O$$

being for arabinose about 75 per cent and for xylose about 90 per cent of the theoretical. Yet by making the distillation under carefully controlled conditions, it is possible, by means of formulas or tables which have been established for different weights of pure pentoses, to make a determination with a very close degree of approximation. (See also p. 922.)

Different reagents have been used for precipitating the furfural in the determination of pentoses. Tollens and Stone first attempted to determine furfural by precipitating with ammonia as furfuramide. An important advance was then made by Tollens, in company with Günther, de Chalmot, Flint, and Mann, in using phenylhydrazine for precipitating the furfural. The use of phenylhydrazine was attended, however, with certain inconveniences and was finally abandoned upon the discovery by Councler[25] of the precipitating action of phloroglucinol. The phloroglucinol method, as first developed by Tollens and Krüger,[26] was further improved by Tollens and Rimbach, and finally established in its present form by Tollens and Kröber.[27]

Description of the Method. The necessary apparatus for making the determination is shown in Fig. 293. From 2 to 5 g. of substance, according to the richness of the material in pentoses or pentosans, is placed in a 300-ml. distillation flask with 100 ml. of hydrochloric acid of 1.06 sp. gr. The flask is closed with a two-hole rubber stopper, one opening of which is fitted to the connecting tube of a condenser and the other to a small separatory funnel. The latter is preferably of cylindrical form with graduation marks at 30 ml. and 60 ml. The flask is then placed in a bath of Rose's alloy (1 part lead, 1 part tin, and 2 parts bismuth, melting near 100° C.), which, after heating just beyond the point of fusion, is brought up slightly above the level of the bottom of the flask. The distillate is received in a graduated cylinder; when 30 ml. of liquid have passed over, which should require 10 minutes, 30 ml. more of the hydrochloric acid of 1.06 sp. gr. are added from the separatory funnel. The process is continued in this way until a drop of the distillate shows no pink coloration with aniline acetate paper (see p. 706). From 9 to 12 portions of 30 ml. usually require to be distilled

[25] *Chem. Ztg.*, 17, 1743 (1893); 18, 966 (1894).
[26] *Z. Ver. deut. Zucker-Ind.*, 46, 21, 195 (1896).
[27] *J. Landw.*, 48, 355 (1900); 49, 7 (1901).

over, depending upon the amount of furfural. The distillation is then suspended and the furfural determined by precipitation with phloroglucinol.

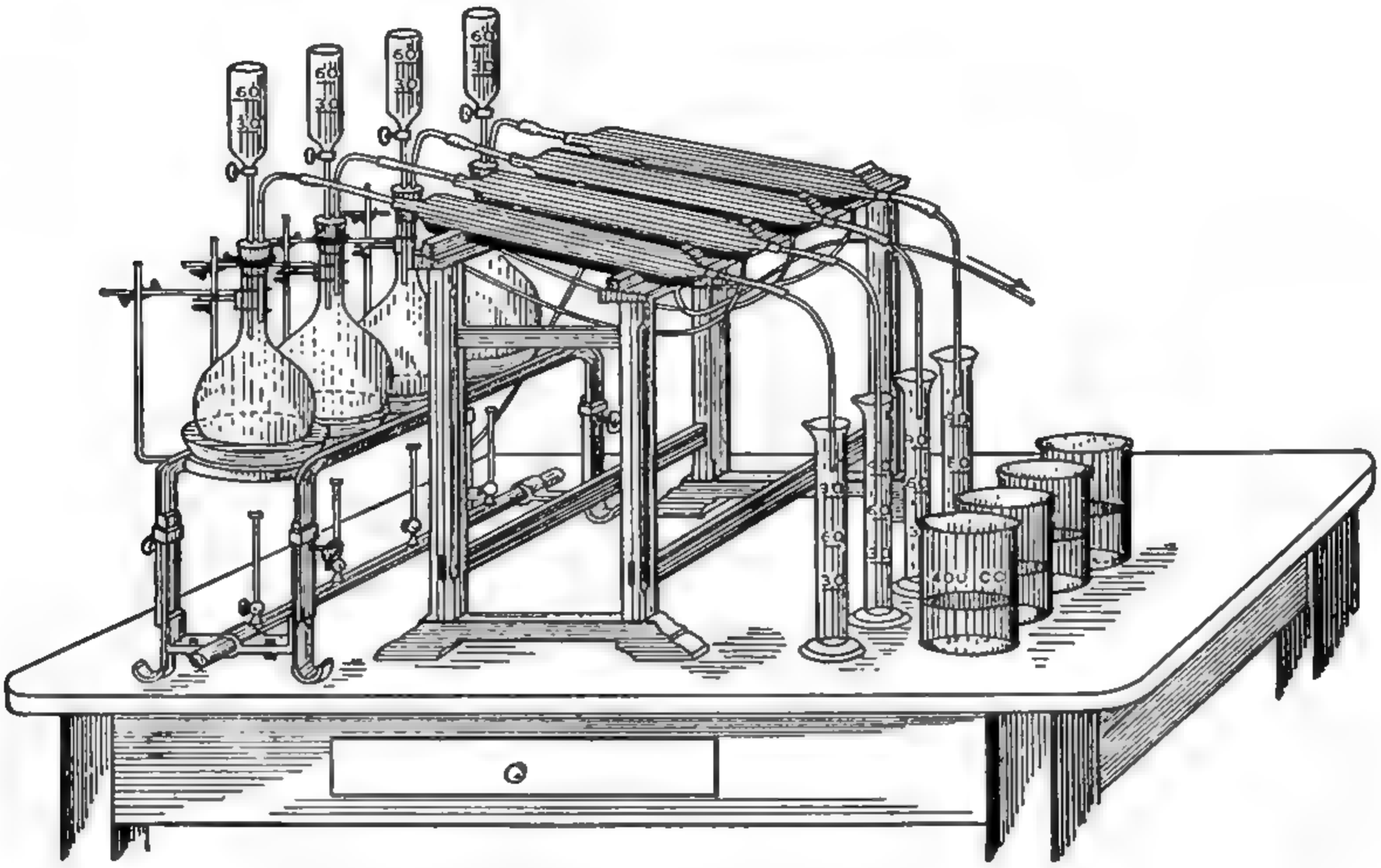


FIG. 293. Apparatus for determining pentoses and pentosans by distillation with hydrochloric acid.

Purification of Phloroglucinol.[28] Dissolve a small quantity of phloroglucinol in a few drops of acetic anhydride, heat almost to boiling, and add a few drops of concentrated sulfuric acid. A violet color indicates the presence of diresorcinol. A phloroglucinol which gives more than a faint coloration may be purified by the following method:

Heat in a beaker about 300 ml. of hydrochloric acid (sp. gr., 1.06) and 11 g. of phloroglucinol, added in small quantities at a time, stirring constantly until it has almost entirely dissolved. Some impurities may resist solution, but it is unnecessary to dissolve them. Pour the hot solution into a sufficient quantity of the same hydrochloric acid (cold) to make the volume 1500 ml. Allow it to stand at least overnight — better several days — to allow the diresorcinol to crystallize out, and filter immediately before using. The solution may turn yellow, but this does not interfere with its usefulness. In using it, add the volume containing the required amount of phloroglucinol to the distillate.

Precipitation of Phloroglucide. The distillate obtained by the method previously described is treated in a 500-ml. lipped beaker with

[28] " Methods of Analysis, A. O. A. C.," 5th ed., pp. 361–362, 1940.

a measured volume of phloroglucinol solution, so that the amount of phloroglucinol is about double that of the furfural expected. The solution first turns yellow, then green, and finally becomes almost black when the amorphous dark-green precipitate of furfural phloroglucide, $C_{11}H_8O_4$, begins to deposit. The liquid is then made up to 400 ml. with the 12 per cent hydrochloric acid (1.06 sp. gr.) and allowed to stand overnight. The solution, after testing with aniline acetate paper to make sure that all furfural has been precipitated, is filtered through a weighed Gooch crucible; the precipitate of phloroglucide is brought carefully upon the asbestos and washed with 150 ml. of water in such a way that the water is not entirely removed from the crucible until the very last. The crucible is then placed upon a support, so that the bottom is free to the air, and dried for 4 hours in a boiling-water oven; it is then placed in a weighing bottle, cooled in a desiccator, and weighed. The increase in weight is the amount of furfural phloroglucide which is calculated to furfural, pentose, or pentosan according to the table of Kröber (Appendix, Table 31).

The weights of pentose in Kröber's table are the averages of the corresponding weights of xylose and arabinose. The weights of pentosan are obtained by multiplying the corresponding weights of pentose by the factor 0.88, which represents the ratio of $n C_5H_{10}O_5$ to $(C_5H_8O_4)_n$ or $\frac{132}{150}$. The table of Kröber has a range for weights of phloroglucide between 0.030 and 0.300 g. For weights of phloroglucide outside of these limits Kröber gives the formulas:

For weight of phloroglucide a under 0.03 g.

$$\begin{aligned}
\text{Grams of furfural} &= (a + 0.0052) \times 0.5170 \\
\text{Grams of arabinose} &= (a + 0.0052) \times 1.1108 \\
\text{Grams of xylose} &= (a + 0.0052) \times 0.9205 \\
\text{Grams of pentoses} &= (a + 0.0052) \times 1.0170 \\
\text{Grams of araban} &= (a + 0.0052) \times 0.9773 \\
\text{Grams of xylan} &= (a + 0.0052) \times 0.8097 \\
\text{Grams of pentosans} &= (a + 0.0052) \times 0.8949
\end{aligned}$$

For weight of phloroglucide a over 0.300 g.

$$\begin{aligned}
\text{Grams of furfural} &= (a + 0.0052) \times 0.5180 \\
\text{Grams of arabinose} &= (a + 0.0052) \times 1.0928 \\
\text{Grams of xylose} &= (a + 0.0052) \times 0.9122 \\
\text{Grams of pentoses} &= (a + 0.0052) \times 1.0026 \\
\text{Grams of araban} &= (a + 0.0052) \times 0.9617 \\
\text{Grams of xylan} &= (a + 0.0052) \times 0.8028 \\
\text{Grams of pentosans} &= (a + 0.0052) \times 0.8824
\end{aligned}$$

The factor 0.0052 represents the weight (5.2 mg.) of phloroglucide, which remains dissolved in the 400 ml. of acid solution.

For weights of phloroglucide which exceed 0.5 g. it may be found necessary to dry for a longer period than 4 hours in order to attain constancy in weight. It is always better in making the determination to regulate the weight of material so that the amount of phloroglucide falls within the range of the table.

Hockett, Guttag, and Smith[29] have extended the Tollens and Kröber method to the determination of lyxose and *d*-ribose, with the following results, expressed in the same way as for the pentoses listed above:

Grams of lyxose = $(a + 0.0052) \times 0.9674$, for a under 0.03 g.
Grams of lyxose = $(a + 0.0052) \times 1.0977$, for $a = 0.03$ to 0.30 g.
Grams of ribose = $(a + 0.0052) \times 0.9664$, for $a = 0.05$ to 0.15 g.

According to the same authors the relation between grams of sugar and grams of phloroglucide a found experimentally is expressed more accurately by these formulas:

$$\begin{aligned} \text{Grams of arabinose} &= 0.0076 + 1.089\,a \\ \text{Grams of xylose} &= 0.0053 + 0.911\,a \\ \text{Grams of lyxose} &= 0.0052 + 1.133\,a \\ \text{Grams of ribose} &= 0.0036 + 1.027\,a \end{aligned}$$

Precautions and Limitations. In making the determination of pentosans by the method of acid distillation, several precautions should be noted. It is important first that the heat be applied to the flask in such a way that charring of solids upon the surface of the glass above the liquid is avoided. Such charring is very likely to occur when the flask is heated over the open flame or upon wire gauze; for this reason the use of the metal bath for heating is to be preferred. The boiling period of 10 minutes for each fraction should be adhered to as closely as possible, and the speed of distillation be kept constant. It is also important that the distillate be perfectly clear and free from suspended impurities before the solution of phloroglucinol is added. With substances which contain much oil or wax, fatty decomposition products are sometimes carried over into the distillate; in determining pentoses in the urine of herbivorous animals, benzoic acid (a decomposition product of hippuric acid) is distilled over in considerable amount. In all such cases the distillate must be filtered from suspended matter before precipitating the furfural with phloroglucinol.

Furfural is quite volatile at ordinary temperature, and some of it may be lost during the long distillation process. This loss may be reduced to a minimum by covering the receiving cylinder with a glass

[29] Paper presented at the Cincinnati meeting of the American Chemical Society, April, 1940; and private communication from Dr. Hockett.

plate with a hole in the center through which the lower end of the condenser passes. Since the phloroglucide is very hygroscopic, the acid in the desiccator must be renewed frequently.

Several important limitations of the distillation method for determining pentoses should be mentioned. (1) Furfural is formed from other substances besides pentoses. (2) Other substances which form a precipitate with phloroglucinol are distilled over besides furfural. (3) Furfural, when boiled with hydrochloric acid in the presence of tannin or lignin, forms insoluble condensation products. (4) The furfural formed is partly decomposed during the distillation.

Furfural from Substances Other Than Pentoses or Pentosans. The formation of furfural from hexuronic acids and from oxycellulose, which also contains carboxyl groups, has already been referred to. Ascorbic acid also yields furfural. Therefore, if pentoses or pentosans are to be determined in animal or plant materials, the presence of these interfering substances must be taken into consideration. Methods for the determination of hexuronic acids in the presence of pentoses or pentosans are described on p. 924, and for that of ascorbic acid on p. 928.

In order to apply a correction to the total phloroglucide obtained from combinations of pentoses and the other substances just mentioned, the quantity of furfural phloroglucide corresponding to a given weight of each of them must be known.

Ehrlich and Schubert[30] found that 2.64 parts of galacturonic acid, upon distillation with hydrochloric acid and precipitation with phloroglucinol, give 1 part of furfural phloroglucide. Therefore, the weight of galacturonic acid found by an independent method is divided by 2.64. the phloroglucide thus found subtracted from the total phloroglucide, and the weight of pentose corresponding to the difference found from Kröber's table.

Myers and Baker[31] have observed, however, that the factor 2.64 is not constant, but varies with the quantity of galacturonic acid as shown in the third column of Table CXXI.

Myers and Baker also call attention to the fact that Ehrlich did not take into consideration the solubility of the phloroglucide in the 400 ml. of 12 per cent hydrochloric acid, which is 0.0052 g. If this correction is added to the weight of the phloroglucide, the ratio of galacturonic acid to phloroglucide changes to the figures given in the fourth column of the table. It is noted that these factors also vary, in the opposite direction to those in column 3, but to a much smaller extent.

In order to obtain more accurate results than is possible by Ehrlich's

[30] *Ber.*, 62, 1974 (1929).
[31] *Delaware Agr. Expt. Station, Bull.* 187, 1934.

TABLE CXXI

Galacturonic Acid, $C_6H_{10}O_7$	Furfural Phloroglucide	Ratio of Acid to Uncorrected Phloroglucide	Ratio of Acid to Corrected Phloroglucide
g.	g.		
0.0631	0.0211	2.990	2.400
0.1201	0.0443	2.711	2.426
0.1684	0.0632	2.644	2.462
0.2129	0.0806	2.641	2.481
0.2590	0.0982	2.637	2.505
0.3048	0.1157	2.634	2.521

procedure, the solubility of the phloroglucide should be considered by the use of the factors given in column 4 of the table. But then it is necessary to consider it also in the calculation of the pentose from the difference in the weights of the phloroglucides, that is 0.0052 g. must be added to the weight of the total phloroglucide found. Then the weight of the phloroglucide equivalent to that of the galacturonic acid, calculated by the factors in column 4 of the table, is subtracted from the corrected total weight of phloroglucide, and the difference is converted into the equivalent weight of pentose. If the difference is less than 0.03 g., it is multiplied by the factors shown on p. 907, 1.1108 for arabinose, 0.9205 for xylose, etc.; it is to be noted that these factors refer to the corrected weight of phloroglucide, $a + 0.0052$, and are therefore directly applicable. If the difference is greater than 0.03 g., the Kröber table is used, but since this is based not on $a + 0.0052$, but on a, 0.0052 must be subtracted from the difference, and the corresponding weight of pentose found from the table.

Phloroglucide factors, similar to those reported by Ehrlich and by Myers and Baker for galacturonic acid, have not been determined for glucuronic or mannuronic acid. Lefèvre and Tollens found that 1 part of phloroglucide corresponds to 3 parts of glucuronic acid lactone or 3.31 parts of glucuronic acid, but confirmation of this figure is lacking.

Substituted Furfurals. The distillation of other products besides furfural which give precipitates with phloroglucinol has long been recognized. Methylfurfural, which is obtained by the distillation of methylpentoses with hydrochloric acid, forms for example a red precipitate with phloroglucinol, which, unless removed by solution in alcohol, as afterwards described, will give too high a weight of furfural phloroglucide. In the same way hydroxymethylfurfural, which is formed in slight amounts by the action of hydrochloric acid upon fructose, sucrose, and other hexose carbohydrates, forms a precipitate with phloroglucinol.

Effect of Tannin and Lignin. Sakostschikoff, Iwanowa, and Kurennowa[32] found that, when substances containing tannin, as cottonseed hulls, are analyzed by the Tollens method, low results are obtained. This is due to the fact that under the conditions of the procedure the tannin condenses with the furfural. Lignin also forms such condensation products but on the other hand yields formaldehyde when boiled with hydrochloric acid. The formaldehyde gives a precipitate with phloroglucinol, and the second reaction would therefore tend to give high results. To obviate the effect of tannin, the above-named authors extracted the tannin by heating with water in an autoclave for 2 hours under 1.5 atmospheres' pressure. The residue was heated for 2 hours more with 4 per cent sulfuric acid at 2 atmospheres' pressure, to hydrolyze the pentosans to pentoses. The acid extract and the final residue were then analyzed by the Tollens method, and the sum of the pentosans was found to be 22.28 per cent of the original hulls, while a direct analysis of the hulls gave only 12.02 per cent. Even the higher figure probably does not represent the actual pentosan content, because pentosan could not be determined in the water extract containing the tannins, and any lignin in the final residue may have affected the pentosan determination in that material. Similar observations have been recorded by Hilpert and Meybier.[33] An exact method for determining pentosans in the presence of lignin or tannin has yet to be devised.

Destruction of Furfural. The fact that pentoses and pentosans do not usually yield the theoretical quantity of furfural upon distillation with hydrochloric acid has been explained by the partial destruction of the furfural. Several authors, such as Jolles,[34] Pervier and Gortner,[35] and Youngburg,[36] have proposed distillation in a current of steam to prevent this decomposition, and have reported practically quantitative results by this procedure. But others, as Iddles and Robbins,[37] and Kline and Acree,[38] found only a small or no increase in the furfural recovered. The last-named authors ascribe the low yields in the analysis of natural products to the presence of lignin (see above) rather than to the destruction of furfural.

Kline and Acree also observed that if nitrates or nitric acid are present with pentoses, as for instance when nitric acid is used for the hydrolysis of the pentosans, the nitric acid or the chlorine evolved by in-

[32] *Ind. Eng. Chem., Anal. Ed.*, 6, 205 (1934).
[33] *Ber.*, **71B**, 1962 (1938).
[34] *Sitzungsber. Wiener Akad.*, 114 (II b), 1191 (1905).
[35] *Ind. Eng. Chem.*, 15, 1255 (1923).
[36] *J. Biol. Chem.*, 73, 599 (1927).
[37] *Ind. Eng. Chem., Anal. Ed.*, 5, 55 (1933).
[38] *Bur. Standards J. Research*, 8, 25 (1932).

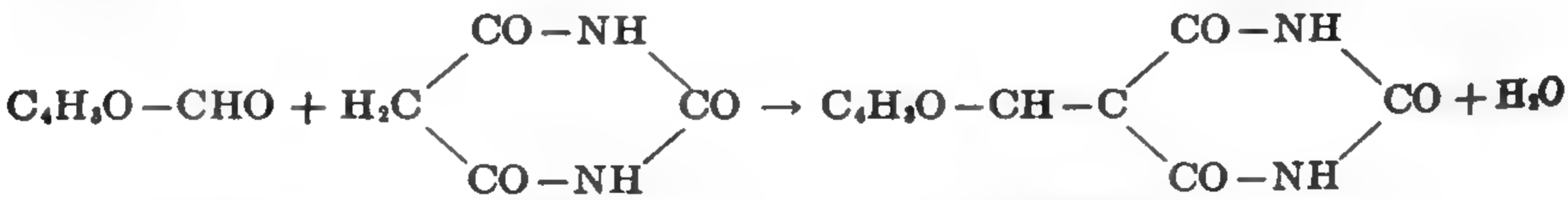
teraction with the hydrochloric acid during the distillation oxidizes the furfural and causes low yields. In such cases the nitric acid must first be removed by precipitation with nitron, and the filtrate used for the furfural distillation.

The Tollens and Kröber method has been critically studied and further standardized by Angell, Norris, and Resch,[39] who also give new equations for the weight of different pentoses and uronic acids corresponding to given weights of phloroglucide. But the older Kröber tables are still in most general use.

Precipitation of Furfural by Means of Barbituric Acid. Jäger and Unger[40] have suggested barbituric acid for precipitating furfural in the presence of foreign distillation products. The hydroxymethylfurfural formed from hexoses, sucrose, starch, cellulose, and other hexose saccharides, though reacting with phloroglucinol, forms a precipitate with barbituric acid only when present in very high concentrations not met with in practice. Methylfurfural, however, is precipitated by barbituric acid (see p. 920).

In the barbituric acid method, as modified by Peter, Thaler, and Täufel,[41] a quantity of material containing about 1.5 g. of pentosan is distilled as in the Tollens and Kröber procedure, but only 7 fractions of 30 ml. each need be distilled, the later fractions containing no furfural but only hydroxymethylfurfural. One-half gram of pure barbituric acid is dissolved in 25 ml. of 12 per cent hydrochloric acid, the solution filtered if necessary, and then added to the distillate. The mixture is allowed to stand for at least 18 hours, and the precipitate filtered off through a Gooch crucible or a Jena fritted glass crucible (1 A G, 3/5-7). It is washed twice with water, dried to constant weight at 130° C., and quickly weighed in a closed vessel, because it is very hygroscopic.

The reaction between furfural and barbituric acid proceeds as follows:



Furfural (96) — Barbituric acid (128) — Condensation product (206)

Unlike phloroglucinol, barbituric acid combines with furfural in stoichiometric proportion, 100 parts of condensation product corresponding to 46.6 parts of furfural.

[39] *Biochem. J.*, **30**, 2146 (1936).
[40] *Ber.*, **35**, 4440 (1902); **36**, 1222 (1903).
[41] *Z. Untersuch. Lebensm.*, **66**, 143 (1933).

The furfural is calculated from the condensation product by the formula:

$$\text{Grams furfural} = 46.6\ (N + 0.0000122\ L)$$

where N is the weight of condensation product in grams, and L the total volume of the liquid in milliliters, 0.0000122 representing the solubility of the precipitate. According to Peter, Thaler, and Täufel, the error in the furfural amounts to ±0.3 per cent. They also found that galactose, lactose, or mannose, when present, reduces the quantity of furfural recovered from the pentoses.

Precipitation of Furfural by Means of Thiobarbituric Acid. Dox and Plaisance,[42] who have also studied the method of Jäger and Unger, found that a large excess of barbituric acid must be used in order to obtain quantitative results, but if thiobarbituric acid is used instead, only a small excess over the theoretical amount is required, and the solubility of the precipitate is so slight that no correction need be applied for it. The reaction is perfectly analogous to that with barbituric acid, 100 parts of condensation product corresponding to 43.24 parts of furfural.

To prepare the thiobarbituric acid reagent, 0.25 g. of pure acid is dissolved in 15 ml. of warm, concentrated hydrochloric acid, the solution diluted with water to 50 ml. and filtered. The distillate containing the furfural is treated with a slight excess of the reagent which produces a lemon yellow, flocculent precipitate. The precipitate is allowed to settle overnight, and then transferred to a Gooch crucible with 12 per cent hydrochloric acid. It is washed with water, dried at 100° C., and weighed. The result is multiplied by 0.4324, to obtain the weight of furfural, and the corresponding amount of pentose or pentosan found from Kröber's table. If methylfurfural is present in addition to furfural, it can be calculated approximately from the nitrogen and sulfur content of the precipitate. Hydroxymethylfurfural does not interfere.

Wise and Peterson[43] obtained excellent results by this method in determining arabinose in the presence of galactose, and they have used it successfully in the analysis of arabogalactan from larch wood.

Kline and Acree[44] state that it is better to wash the precipitate with 12 per cent hydrochloric acid instead of water, because it is liable to form a colloidal solution in water and run through the filter.

A micromethod based on that of Dox and Plaisance has been described by Bailey.[45]

[42] *J. Am. Chem. Soc.*, 38, 2156 (1916).
[43] *Ind. Eng. Chem.*, 22, 362 (1930).
[44] *Bur. Standards J. Research*, 8, 25 (1932).
[45] *Ind. Eng. Chem., Anal. Ed.*, 8, 389 (1936).

Dinitrophenylhydrazine has also been used for the gravimetric determination of furfural, but it is necessary to operate at 0° C. to obtain quantitative precipitation.[46]

Volumetric Furfural Determination. As early as 1890 Günther and Tollens[47] proposed to titrate the furfural in the distillate with phenylhydrazine, using aniline acetate as the indicator of the end point, but the results obtained with this method were not satisfactory. Other volumetric procedures have been suggested from time to time.[48] Pervier and Gortner[49] found that furfural can be estimated in acid solution by oxidation with bromate in the presence of bromide, the end point being determined electrometrically. But subsequent investigators encountered difficulties in fixing the end point, and erratic results were often obtained. Powell and Whittaker[50] were able to overcome this difficulty by employing an excess of bromate, adding potassium iodide, and titrating back with thiosulfate. The oxidizing reagent is prepared by dissolving 3 g. of potassium bromate and 50 g. of potassium bromide to 1 liter. The distillate containing the furfural is diluted in a volumetric flask to 500 ml. with 12 per cent hydrochloric acid. Twenty-five milliliters of the bromate-bromide reagent is pipetted into a glass-stoppered flask, and 200 ml. of the diluted furfural distillate is added. A blank is prepared with 200 ml. of 12 per cent hydrochloric acid. The flasks are placed in the dark for 1 hour. Then 10 ml. of 10 per cent potassium iodide solution is added to each flask, and the excess iodine titrated back with 0.1 *N* thiosulfate solution. Each milliliter of the difference between the two titrations is equivalent to 0.0024 g. furfural.

This method gives good results even with as little as 2 to 3 mg. of furfural, where the precipitation methods do not reveal its presence or give low results. The whole operation can be completed in a little over an hour after the distillation is finished, whereas the gravimetric methods require at least another day.

If hydroxymethylfurfural is present in the distillate this may be destroyed by redistilling with 12 per cent hydrochloric acid saturated with sodium chloride, according to Schmidt-Nielsen and Hammer.[51] Since some of the furfural is also decomposed during the second distillation, the result must be increased by 3.3 per cent.

[46] Simon, *Biochem. Z.*, **247**, 171 (1932); Reynolds, Osburn, and Werkman, *Iowa State Coll. J. Sci.*, **7**, 443 (1933). See also p. 920.

[47] *Ber.*, **23**, 1751 (1890).

[48] See bibliography in article by Pervier and Gortner (Ref. 49).

[49] *Ind. Eng. Chem.*, **15**, 1255 (1923).

[50] *J. Soc. Chem. Ind.*, 43, 35T (1924).

[51] *Kgl. Norske Videnskab. Selskab. Forh.*, **5**, 84 (1932).

As another volumetric procedure that of Noll and Belz[52] may be mentioned. The furfural distillate is first neutralized with sodium hydroxide, and a measured quantity of a solution of hydroxylamine hydrochloride is added, in excess of that required to form α-furanaldoxime and free hydrochloric acid. The latter is titrated with 0.1 *N* sodium hydroxide. A blank is run with hydroxylamine hydrochloride alone, and the titer deducted from that of the furfural distillate. One milliliter of 0.1 *N* sodium hydroxide is equivalent to 0.0096 g. of furfural. The process has not yet been tried sufficiently by other chemists to form a conclusion as to its reliability.

Colorimetric Furfural Determination. When only very small amounts of pentoses are to be determined, as in the analysis of urine, colorimetric methods are to be preferred.

Method of McCance.[53] This is based on the deep crimson-violet color which furfural produces with benzidine. One-half gram of this reagent is dissolved in 100 ml. of equal volumes of absolute alcohol and glacial acetic acid, and the solution filtered. The standard is prepared by placing 3 ml. of a 0.1 per cent solution of the sugar to be determined, e.g., arabinose, in a test tube, followed by the addition of 3 ml. of water and 3 ml. of concentrated hydrochloric acid. Similar test tubes are prepared with 1, 2, and 3 ml. of the solution to be analyzed, to which is added enough water to make up to 6 ml., and then 3 ml. of concentrated hydrochloric acid. After thorough mixing, each test tube is provided with a long glass tube to act as a reflux condenser, and the test tubes are then placed for 2 hours in a boiling-water bath. The tubes are quickly cooled, 4 ml. of benzene is added to each, and the tubes are vigorously shaken to extract the furfural. After 30 minutes' standing, 2 ml. of the benzene layer is pipetted off from each tube and transferred to dry test tubes. Four milliliters of the benzidine reagent is then added to each tube and the contents mixed. After standing for at least 30 minutes, but not more than 2 hours, the unknowns are compared colorimetrically with the standard which contains the furfural from 1.5 mg. of pentose. For accurate results it is necessary that the material to be analyzed contain no coloring matter soluble in benzene, or buffer salts which would reduce the strength of the hydrochloric acid.

Method of Youngburg. McCance's method has been criticized by Youngburg[54] on the ground that some of the furfural is destroyed during the long heating period with acid. Youngburg's apparatus consists of a large test tube with a three-hole stopper. Through one of the holes

[52] *Papier-Fabr.*, 29, Tech. Wiss. Teil 33–4 (1931).
[53] *Biochem. J.*, 20, 1111 (1926).
[54] *J. Biol. Chem.*, 73, 599 (1927).

passes a thermometer reaching nearly to the bottom of the tube, through the second an inlet tube for steam, also ending near the bottom, and through the third a short outlet tube which is connected through a condenser with the receiver which is a test tube graduated at the 10-ml. mark. The solution to be analyzed and containing from 0.1 to 1 mg. of pentose or pentosan is placed in the distilling tube. Three milliliters of 85 per cent phosphoric acid is added, and the tube is heated to 125° C. It is then connected with the steam generator and the condenser, and the distillation carried out at a temperature of 175° C. (not over 180° C.). When 10 ml. of distillate has been collected, another receiver is put in place of the first one, and this is repeated until 2 ml. of the distillate, removed from the receiver, does not produce any color with 0.25 ml. of pure aniline and 2 ml. of glacial acetic acid after a period of 30 seconds. Then 2 ml. each of all the previous fractions are mixed together. Five milliliters of the mixed distillates is transferred to a 10-ml. graduated tube, and 5 ml. of a standard solution containing 0.05 mg. furfural is placed in another such tube. Then 0.5 ml. aniline and 4 ml. glacial acetic acid are added to the sample and to the standard, the volumes are completed to 10 ml., and the tubes are allowed to stand in the dark for 15 minutes. The sample is compared colorimetrically with the standard within 40 minutes from the time at which the aniline and acetic acid were added. The following factors are used to convert furfural found into its equivalent of various pentoses:

$$d\text{-Xylose} = \text{Furfural} \times 1.56$$
$$d\text{-Ribose} = \text{Furfural} \times 2.00$$
$$d\text{- or } l\text{-Arabinose} = \text{Furfural} \times 2.40$$
$$d\text{-Lyxose} = \text{Furfural} \times 3.00$$

Under the conditions of the test, hexoses and hexose saccharides yield small quantities of hydroxymethylfurfural which also gives a color reaction with aniline acetate, but usually the errors produced from this source can be neglected.

The acid distillation method for determining pentoses gives good results with pure arabinose or xylose but, as has been shown, yields only rough approximations in the case of the various complex materials yielding furfural. Even in the case of pure pentosans the calculation of furfural to a mixture of araban or xylan in equal amounts, when perhaps the pentosan itself may consist almost entirely of one substance, may involve an error of several per cent in the calculation. In certain plant exudations, as cherry gum, the pentosans consist almost entirely of araban; in the hemicelluloses of certain woods, as the beech, almost entirely of xylan; in the encrusting substances of most cellular tissues, of

variable mixtures of araban and xylan. Until accurate methods are available for the estimation of xylan and araban in the presence of interfering substances, the calculation of furfural to a mixture of xylan and araban in equal amounts can be regarded only as a conventional approximation.

Applications of Pentosan Method. The determination of pentosans, notwithstanding certain limitations of the method, has found numerous applications in the assay of plant gums, in the analysis of feeding materials,[55] in the examination of forestry products, and in other ways. A single example of such application is given in the analysis of paper stock. Kröber,[56] for example, gives the following determination of pentosans in different raw materials used in paper manufacture.

TABLE CXXII

Material	Pentosans Calculated to Ash-Free Dry Substance
	per cent
Mechanical wood pulp	12.24
Mechanical wood pulp	11.93
Cotton	1.03
Linen	2.20
Bleached straw	26.76
Bleached raw cellulose (soda process)	6.41
Bleached raw cellulose (sulfite process)	7.09

An application of the above results to a special problem which may confront the paper chemist is taken from the work of Tollens.[57]

A sample of newspaper is known to be made up of mechanical wood pulp and sulfite cellulose; it is desired to know the percentages of each which were used. The sample of paper upon analysis showed 10 per cent pentosans calculated to ash-free dry substance. Calling the percentage of pentosans in the ash-free dry substance of mechanical wood pulp 12 per cent and of sulfite cellulose 7 per cent, then

$$\frac{10 - 7}{12 - 7} \times 100 = 60 \text{ per cent mechanical wood pulp}$$

$$\frac{12 - 10}{12 - 7} \times 100 = 40 \text{ per cent sulfite cellulose}$$

[55] Browne and Beistle, *J. Am. Chem. Soc.*, 23, 229 (1901).
[56] *J. Landw.*, 49, 7 (1901).
[57] *Papier-Ztg.*, 26, 17 (1907).

For other applications of the method the chemist is referred to the original paper by Tollens.

Determination of Pectin by the Furfural Method. The formation of furfural from galacturonic acid upon distillation with acid has already been mentioned. Since pectin contains pentosan and galacturonic acid, Silin and Silina[58] have proposed a modification of the Tollens method for its determination in beet products. The furfural obtained is calculated to pectin by means of empirical factors which have been determined by Silin and Silina. Five milliliters of juice, sirup, etc., containing 0.001 to 0.015 g. pectin, is measured into a 100-ml. round-bottom flask, and 2.5 ml. of concentrated hydrochloric acid plus 32.5 ml. of hydrochloric acid of sp. gr. 1.06 are added, making a total volume of 40 ml. If the sample material is dry, 40 ml. of hydrochloric acid, sp. gr. 1.06, is used. The flask is connected with an air condenser, consisting of a glass tube, 9 mm. in diameter and 60 to 70 cm. long, bent downward. The distillate is collected in a 50-ml. graduated cylinder. The flask is placed on an asbestos plate with a circular hole, 3 to 3.5 cm. in diameter, and covered with wire gauze. The solution is distilled slowly and uniformly, at a temperature not exceeding 125° C., until at the end of 3 hours 30 ml. of distillate has been collected. The distillate is transferred quantitatively to a 100-ml. volumetric flask, neutralized with dilute (10 per cent) sodium hydroxide solution, phenolphthalein serving as indicator, and made up to volume. The solution is filtered, and the furfural determined colorimetrically with aniline acetate, as described in the method of Youngburg (p. 915), by comparison with freshly prepared furfural standards. The furfural from the pectin must be corrected for hydroxymethylfurfural formed from sucrose, by deducting 0.00125 g. furfural for each gram of sucrose present in the sample. The corrected furfural is multiplied by 3.8 to convert it into pectin present in press or diffusion juice. Treatment with lime changes the nature of the pectin, and for this reason the factor 5.5 must be used for limed and saturated juices, and all the later products of the sugar factory.

This method has been found by Baerts and Vanderwijer[59] to give reproducible results. However, they correctly point out that furfural may be formed from other beet constituents besides pectin and sucrose, and they propose the designation "furfurogenic substances" instead of "pectin." They also observed[60] that a mixture of pectin and sucrose yields more furfural than the total furfural obtained when either of them is distilled separately.

[58] *Z. Ver. deut. Zucker-Ind.*, **83**, 390 (1933).
[59] *Sucr. belge*, **55**, 165 (1936).
[60] *Fourth Congr. intern. tech. chim. ind. agr.*, Bruxelles, 3, 127 (1935).

DETERMINATION OF METHYLPENTOSES AND METHYLPENTOSANS

The conversion of methylpentoses into methylfurfural by distillation with hydrochloric acid was described on p. 708. The method for determining methylpentoses, or methylpentosans, is based upon determining the amount of methylfurfural which is thus produced. The details of the method, which were first worked out by Tollens and Ellett[61] and further elaborated by Tollens and Mayer[62] are practically the same as described for the determination of the pentoses. The same apparatus (Fig. 293) is used, and the substance is distilled with 12 per cent hydrochloric acid (1.06 sp. gr.) until a drop of the distillate gives no yellow coloration with aniline acetate paper. The methylfurfural is then precipitated with phloroglucinol and the solution allowed to remain overnight, when the red precipitate of methylfurfural phloroglucide is filtered, washed, dried, and weighed in exactly the same manner as described for furfural phloroglucide.

The weight of methylfurfural phloroglucide is then calculated either to rhamnose by the table of Ellett and Tollens or to fucose by the table of Mayer and Tollens. The rhamnose, $CH_3C_5H_9O_5 \cdot H_2O$, is calculated to rhamnosan $(CH_3C_5H_7O_4)_n$ by multiplying by the factor $\frac{144}{180} = 0.80$; and the fucose, $CH_3C_5H_9O_5$, to fucosan by the factor $\frac{144}{164} = 0.89$. The combined table giving the weights of rhamnose, rhamnosan, fucose, fucosan, and methylpentosan (mixture of equal parts rhamnosan and fucosan) corresponding to different weights of methylfurfural phloroglucide is given in the Appendix (Table 32).

Instead of the tables the following formulas may be used in which Ph is the weight in grams of methylfurfural phloroglucide.

$$\text{Fucose} = 2.66\ \text{Ph} - 12.25\ \text{Ph}^2 + 0.0005$$
$$\text{Rhamnose} = 1.65\ \text{Ph} - 1.84\ \text{Ph}^2 + 0.0100$$
$$\text{Methylpentosan} = 1.85\ \text{Ph} - 6.25\ \text{Ph}^2 + 0.0040$$

To calculate methylfurfural, Fromherz[63] gives the formula

$$\text{Methylfurfural} = 0.5263\ (\text{Ph} + 0.000018\ n)$$

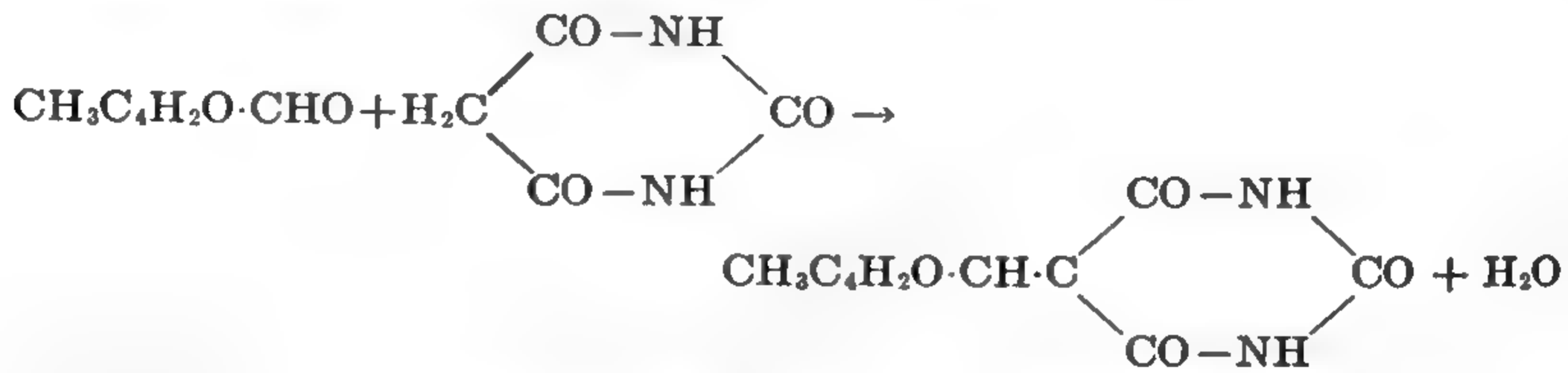
where n is the volume of solution in milliliters. According to Iddles and French[64] the following formula is more satisfactory:

$$\text{Methylfurfural} = 0.4780\ (\text{Ph} + 0.0199)$$

[61] *Ber.*, 38, 492 (1905).
[62] *Z. Ver. deut. Zucker-Ind.*, 57, 620 (1907); *Ber.*, 40, 2441 (1907).
[63] *Z. physiol. Chem.*, 50, 241 (1906/07).
[64] *Ind. Eng. Chem., Anal. Ed.*, 8, 283 (1936).

Fucose decomposes more slowly than rhamnose with hydrochloric acid, so that the distillation must be continued longer. Consequently more decomposition products of methylfurfural are formed in distilling fucose, with a correspondingly less yield of phloroglucide.

Methylfurfural, according to Fromherz,[65] may also be estimated by precipitation with barbituric acid in the same manner as described for furfural. The reaction takes place according to the equation:



Methylfurfural (110) Barbituric acid (128) Condensation product (220)

Two parts of condensation product thus correspond to exactly one part of methylfurfural. The yellow crystalline precipitate is filtered in a Gooch crucible, washed with water, and then dried for 5 hours in a steam bath. The precipitate is then weighed and, after correcting for its slight solubility in the 12 per cent hydrochloric acid (2.29 mg. in 100 ml.), calculated to methylfurfural by dividing by 2.

The thiobarbituric acid method of Dox and Plaisance (p. 913) has been applied to the determination of methylfurfural by Iddles and French.[66] After the reagent has been added to the methylfurfural solution, the mixture is allowed to stand for 2 days to complete the precipitation. The weight of precipitate is multiplied by 0.4660 to convert it into that of methylfurfural. The error does not exceed 1 mg.

According to the same authors, methylfurfural may also be precipitated with a saturated solution of dinitrophenylhydrazine in 2 *N* hydrochloric acid (see p. 914), at 0° C. The precipitate is filtered off after 1 hour, washed with 2 *N* hydrochloric acid, and dried over phosphorus pentoxide in a partial vacuum. The weight of the precipitate, multiplied by 0.3793, gives methylfurfural with an error of about 0.2 mg. The volumetric method of Powell and Whittaker (p. 914) did not give satisfactory results with methylfurfural.

DETERMINATION OF PENTOSES AND METHYLPENTOSES IN MIXTURES

Method of Tollens and Ellett. The method of determining pentoses and methylpentoses in mixtures, first worked out by Tollens and El-

[65] *Loc. cit.*

[66] *Ind. Eng. Chem., Anal. Ed.*, **8**, 283 (1936).

lett,[67] is based upon the solubility of methylfurfural phloroglucide and the insolubility of furfural phloroglucide in warm 95 per cent alcohol.

In making the determination the material is distilled with 12 per cent hydrochloric acid, the distillate precipitated with phloroglucinol, and the mixed phloroglucides of furfural and methylfurfural filtered in a Gooch crucible, dried, and weighed according to the usual process.

The crucible containing the mixed phloroglucides is then placed in a small beaker with 95 per cent alcohol which is heated nearly to boiling. The brown-colored solution is then sucked off through the crucible by means of a filter pump, and the extraction with hot 95 per cent alcohol repeated twice more in the same way. The crucible containing the insoluble furfural phloroglucide is then dried for 2 hours in a hot-water oven and reweighed in a weighing bottle. The residual weight of furfural phloroglucide is then calculated to pentoses or pentosans and the loss in weight, due to methylfurfural phloroglucide, calculated to methylpentoses, or methylpentosans, by means of the respective tables or formulas.

Trials of this method of separation upon known mixtures of pentoses with methylpentoses were made by Ellett and Tollens and by Mayer and Tollens with very close agreements.

Modification by Haywood of the Tollens-Ellett Method. Haywood,[68] who has tested the method of Tollens and Ellett, believes that a correction should be made for the slight solubility of the furfural phloroglucide in 95 per cent alcohol. Experiments made by Haywood upon the phloroglucide obtained from pure arabinose showed that for varying weights of substance, and extracting 3 to 5 times with alcohol, a very uniform weight of about 0.0037 g. was always dissolved. Haywood believes the substance thus dissolved to be occluded phloroglucinol and not phloroglucide. The following slight modification of the Tollens-Ellett method is proposed by Haywood:

Place the Gooch crucible containing the mixed phloroglucides in a 100-ml. beaker and pour into the crucible 30 ml. of 95 per cent alcohol heated to 60° C. Place the beaker for 10 minutes in a water bath heated to 60° C. Remove the beaker and crucible, and suck from the crucible all alcohol remaining therein with a suction pump. Repeat this alternate extraction and sucking dry of the precipitate 3 to 5 times, according to the color of the filtrate obtained. After the final extraction place the Gooch crucible in a water oven and dry 4 hours, making the final weighing in a closely stoppered glass weighing bottle.

The difference in weight between the furfural phloroglucide plus

[67] *Z. Ver. deut. Zucker-Ind.*, 55, 19 (1905).

[68] *Bull.* 105, U. S. Bur. Chem., p. 112.

methylfurfural phloroglucide first obtained and the furfural phloroglucide remaining after extraction with alcohol, minus 0.0037, represents the amount of methylfurfural phloroglucide present, from which the methylpentose or methylpentosan is calculated by the tables or formulas.

To obtain the weight of pentosans, subtract the corrected weight of methylfurfural phloroglucide from the weight of the mixture and calculate according to Kröber's tables or formulas.

Later investigators have not been able to obtain satisfactory results by the method of Tollens and Ellett, largely because the procedure is empirical and the results are affected by details in manipulation. Fromherz[69] found that the total amount of phloroglucide precipitate as well as that of the alcohol-soluble portion is influenced by the length of time for which it has stood before filtration; furthermore, the weight of the precipitate does not vary in direct proportion to the weight of sample taken. This has been confirmed by Iddles and French.[70]

A volumetric method by which furfural and methylfurfural may be determined in mixtures of the two has been described by Hughes and Acree.[71] It is based on the difference in the rate of oxidation of furfural and methylfurfural by bromine in normal hydrochloric acid at 0° C. It has not been applied as yet to mixtures of pentoses and methylpentoses, or pentosans and methylpentosans.

Hughes and Acree[72] have also devised a distillation procedure with 12 per cent hydrochloric acid in the presence of sodium chloride, by which furfural or methylfurfural is obtained quantitatively from xylose, arabinose, and rhamnose. An apparatus with glass joints is used because contact of rubber with hot hydrochloric acid vapor produces a volatile substance that reacts with the bromine used for the oxidation of the furfural. The distillation is carried out in a current of steam at about 110° C. under carefully regulated conditions, and the furfural which escapes from the receiver is caught in a trap. Hockett, Guttag, and Smith[73] have obtained quantitative yields of furfural from lyxose by the same method.

DETERMINATION OF HYDROXYMETHYLFURFURAL

The occurrence of hydroxymethylfurfural in commercial invert sugars produced by the usual acid hydrolysis method, and the tests

[69] *Z. physiol. Chem.*, 50, 241 (1906/07).
[70] *Ind. Eng. Chem., Anal. Ed.*, 8, 283 (1936).
[71] *Ind. Eng. Chem., Anal. Ed.*, 9, 318 (1937).
[72] *J. Research Nat. Bur. Standards*, 21, 327 (1938); 23, 293 (1939).
[73] Paper presented at the Cincinnati meeting of the American Chemical Society, April, 1940.

for detecting its presence, have already been referred to. The quantitative determination of hydroxymethylfurfural has been studied by Troje,[74] who described three different methods. The colorimetric procedure utilizes Fiehe's reaction with resorcinol and hydrochloric acid, and comparison with known standards. In the gravimetric method the hydroxymethylfurfural is coupled with phloroglucinol, and the phloroglucide is weighed. In the titrimetric method the hydroxymethylfurfural is treated with an excess of alkaline iodine solution, and after acidification the excess iodine is determined with standard thiosulfate solution. The hydroxymethylfurfural occurring in saccharine products is first extracted with dry ethyl acetate or ether.

Phloroglucide Method of Fiehe and Kordatzki.[75] These authors found that ether extracts of genuine honeys contain substances which are oxidized by iodine in alkaline solution, but give no precipitate when the phloroglucinol method is used. For this reason the latter method should be used for quantitative purposes, since the colorimetric method requires comparisons with pure hydroxymethylfurfural, which is difficult to prepare and unstable. The phloroglucinol method is carried out as follows:

One hundred grams of the product, usually commercial honey, is dissolved in 400 ml. of water. The solution is clarified with 5 ml. of 30 per cent zinc acetate solution and 5 ml. of 15 per cent potassium ferrocyanide solution. The filtrate is extracted in an efficient apparatus 3 times for 4 hours each with 40 to 50 ml. of ether. To the ether extract are added an equal volume of petroleum ether and 10 g. of anhydrous sodium sulfate. The mixture is allowed to stand for 24 hours with occasional shaking. It is then filtered, and the filtrate evaporated at a low temperature. The dry residue is stirred with 20 ml. of water, and the solution filtered. Five milliliters of the solution is mixed with 5 ml. of 32 per cent hydrochloric acid and 40 ml. of a solution of 2.5 g. phloroglucinol in 400 ml. of 16 per cent hydrochloric acid. After 24 hours' standing the phloroglucide is filtered off, washed with 15 to 20 ml. of water added in small portions, and dried for 3 hours at 100° C. It is then kept for 3 hours at room temperature in a dust-free atmosphere, and weighed. The weight of the corresponding hydroxymethylfurfural is found from the following table:

Phloroglucide, mg.	2	4	5	7.5	10	15	20	25	30	34	36
Hydroxymethylfurfural, mg.	2.3	3.3	4.2	5.0	5.9	7.9	9.9	12	14	15.8	16.3

If the phloroglucide method shows the presence of artificial honey

[74] *Z. Ver. deut. Zucker-Ind.*, **75**, 635 (1925).
[75] *Z. Untersuch. Lebensm.*, **56**, 490 (1928); **58**, 69 (1929).

in a commercial product and it is desired to ascertain whether it contains also genuine honey, Troje's iodine method is applied, as follows. To another 5-ml. portion of the water solution obtained from the ether extract add 10 ml. of $N/10$ iodine solution and enough strong sodium hydroxide solution that after dilution to 100 ml. the liquid is 0.5 N in alkali concentration. Let stand for 2 hours, acidify with 3 N sulfuric acid, and titrate the excess iodine with $N/10$ thiosulfate. Run a blank with 5 ml. of water. One milliliter of $N/10$ iodine required for oxidation corresponds to 6.77 mg. hydroxymethylfurfural. If the result thus found is decidedly higher than that obtained by the phloroglucinol method, the presence of genuine honey is indicated.

According to Weiss,[76] *p*-nitrobenzohydrazide may be used instead of phloroglucinol for precipitating the hydroxymethylfurfural; he also states that it is preferable to extract the honey with ethyl acetate rather than with ether.

Schoù and Abildgaard[77] have found that hydroxymethylfurfural shows a strong absorption band in the ultra-violet, with a maximum at wavelength 282.5 mμ. Pure bee honeys give increasing absorption with decreasing wavelength, but no absorption band in the ultra-violet. The hydroxymethylfurfural in artificial honey and in mixtures with bee honey can be estimated by spectrophotometric comparison with standards prepared from pure hydroxymethylfurfural.

DETERMINATION OF HEXURONIC ACIDS

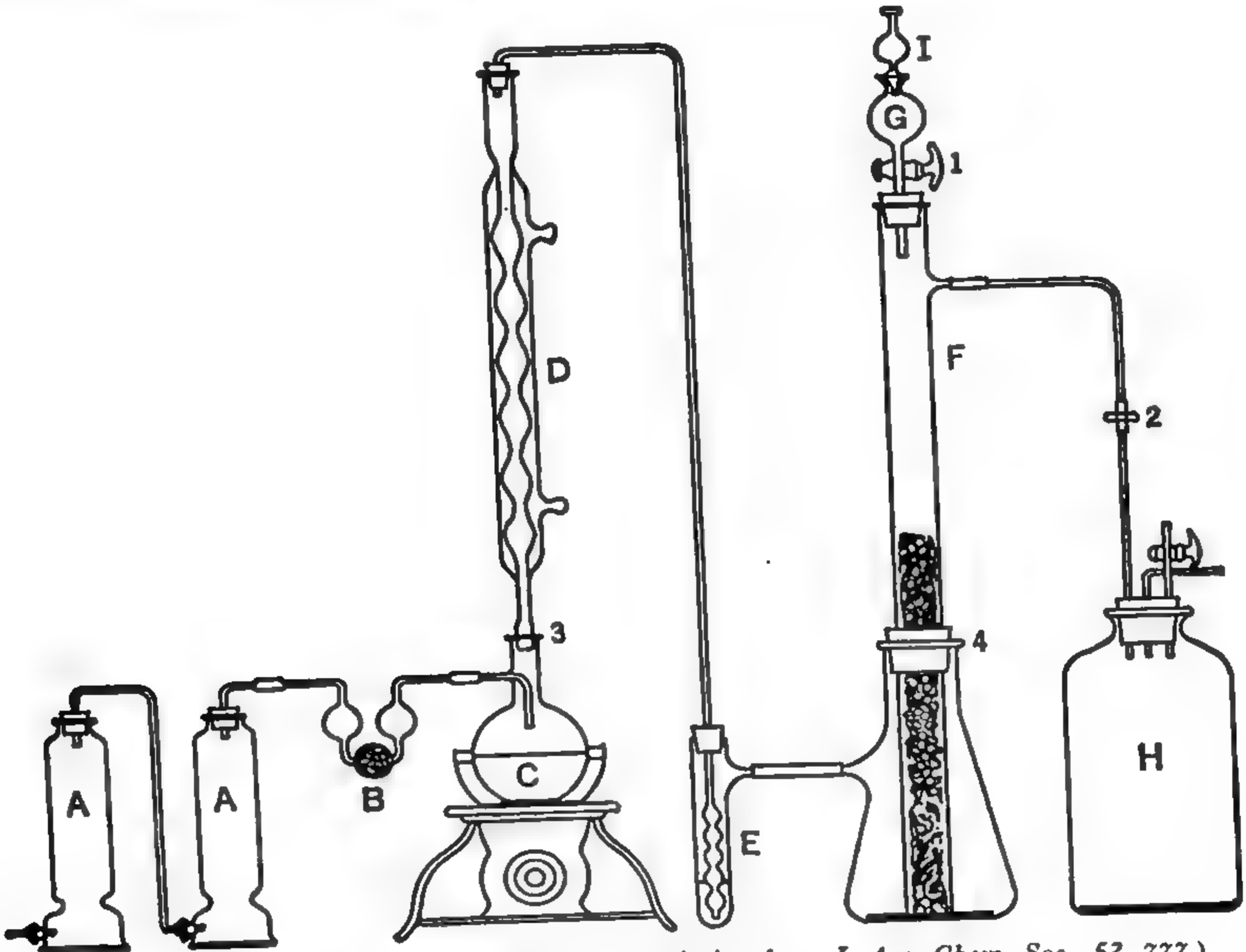
The wide distribution of glucuronic, galacturonic, and mannuronic acids has already been referred to, and it has been shown (p. 909) that when these acids or their high molecular condensation products are heated with acids, as in the Tollens method of pentose estimation, furfural and carbon dioxide are formed according to the equation

$$C_6H_{10}O_7 \longrightarrow C_5H_4O_2 + CO_2 + 3\,H_2O$$

It follows that in the presence of uronic acids the determination of pentoses or pentosans gives too high results. This makes it necessary to estimate the uronic acids by an independent method and to correct the total amount of furfural for that formed from the uronic acids. Lefèvre and Tollens[78] worked out such a method, based on the fact that in the distillation with 12 per cent hydrochloric acid a quantitative yield of carbon dioxide is obtained, according to the above equation.

[76] *Z. Untersuch. Lebensm.*, **58**, 320 (1929).
[77] *Z. Untersuch. Lebensm.*, **68**, 502 (1934).
[78] *Ber.*, **25**, 2569 (1892); **40**, 4153 (1907).

The distillation was carried out in a current of carbon-dioxide-free air, the carbon dioxide absorbed in a potash bulb, and weighed, with the usual precautions observed in elementary organic analysis. The same principle has been retained in all the later modifications of the method. Only one of these will be described.



(Reproduced with permission from J. Am. Chem. Soc., 52, 777.)

FIG. 294. Apparatus for determining uronic acids.

Method of Dickson, Otterson, and Link.[79] In this method the carbon dioxide is not weighed, but measured by titration, after being absorbed by an excess of standard barium hydroxide solution.

Description of Apparatus. This is shown in Fig. 294. The distillation flask *C* is connected by a ground-glass joint, greased with vacuum stopcock grease, to the Allihn reflux condenser *D*. A glass tube leads from the upper end of the condenser to the absorption tube *E* which is filled with 10 per cent silver nitrate solution to remove the last traces of hydrochloric acid, and is connected by a side tube with the Truog absorption tower *F*. This consists of a filtering flask, with side tube, of 500-ml. capacity, in which is inserted, through a rubber stopper, a glass tube of 1-inch diameter and 24 inches high. To make an air-tight joint, the rubber stopper connection is painted over with par-

[79] *J. Am. Chem. Soc.*, 52, 775 (1930).

affin. The bottom of the tower tube should be $\frac{1}{8}$ inch above the inside bottom of the flask. The tube is filled with perforated glass beads to a height of 16 inches. A dropping funnel, of 50-ml. capacity and provided with a soda lime tube, is inserted in the top of the tower tube. A side tube leads from the latter to the 4-liter safety bottle *H* which is connected with a water vacuum pump. At 2 a screw clamp is placed to regulate the air flow. The reaction flask, which is heated in an oil bath, has a side-arm tube reaching below the neck of the flask, to sweep the carbon dioxide liberated in the reaction through the absorption tower by means of air which has been freed from carbon dioxide by being passed through the soda lime bottles *A*. A bulb water trap is placed between these and the reaction flask. All the rubber connections should be made with heavy-wall tubing.

Procedure. The sample, usually 1 to 2 g., depending on the uronic acid content, is transferred to the reaction flask, together with 100 ml. of 12 per cent hydrochloric acid (sp. gr. 1.06). In the case of solutions enough strong hydrochloric acid must be added to bring the acid content to 12 per cent. A few pieces of unglazed porcelain are used to prevent bumping during the distillation. The dropping funnel is filled with a measured quantity of *N*/5 barium hydroxide solution, and the soda lime tube replaced at once. Before the distillation is started, all the carbon dioxide in the system, including that present in the sample in the form of carbonates, must be removed. This is done, according to Phillips, Goss, and Browne,[80] by heating the liquid in the flask, before attaching the condenser, to 70° C. for half an hour, and after replacing the condenser, sweeping a current of carbon-dioxide-free air through the whole apparatus for 20 minutes. The heat is then increased, and, as soon as the solution commences to boil, the barium hydroxide solution is run into the absorption tower. It is washed down with sufficient carbon-dioxide-free water to cover the glass beads. At the same time the velocity of the air current must be increased, to prevent the vapors in the reaction flask from passing back into the soda lime towers. The air current is finally adjusted to about 2 to 3 bubbles per second. The oil bath is now kept at a temperature of 135 to 140° C. for a period of 5 hours to complete the reaction. Screw clamp 2 is then closed, and the absorption tower is disconnected from the silver nitrate trap. Stopcock 1 is opened for a moment to allow the barium hydroxide solution to flow down into the suction flask, and again immediately closed. The side arm of the tower is disconnected, the dropping funnel removed, and the entire contents of the tower are washed into the flask with carbon-dioxide-free water. The excess barium hydroxide is titrated in the

[80] *J. Assoc. Official Agr. Chem.*, **16**, 289 (1933).

flask with $N/10$ hydrochloric acid and phenolphthalein as indicator. The glass beads do not interfere with the titration.

A blank determination is carried out in the same way as described, but without the sample, and the titer previously found is corrected accordingly. Each milliliter of $N/5$ barium hydroxide corresponds to 0.0044 g. carbon dioxide. The percentage of carbon dioxide, on the weight of the sample, multiplied by 4, gives the percentage of uronic acid lactone, since the molecular weight of the latter is 176.

TABLE CXXIII

RESULTS OF DETERMINATIONS OF URONIC ACID IN PLANT MATERIALS

Plant Substance	Uronic Acid Lactone
	per cent
Honeydew melon	3.60
Cantaloupe	4.00
Lima beans	4.20
Peas	4.88
Peeled cucumbers	8.32
Asparagus stalks	9.16
Asparagus tips	9.88
Carrots	10.24
Spinach	10.32
Summer squash	10.64
Cabbage leaves	11.16
Pea pods	11.32
Cucumber peelings	11.96
Cauliflower	12.56
Radish tops	12.72
Eggplant	13.08
Apple peelings	13.16
Kale	14.04
Head lettuce leaves	14.20
Beet tops	14.52
Carrot tops	14.52
Celery (leaves and stalks)	16.72
Orange peelings	17.72

The condenser must be removed from the flask while it is still hot, to prevent sticking. The absorption tower and the glass beads are washed with dilute hydrochloric acid to remove barium carbonate, then with water, and dried, after which they may be reused.

In the analysis of pure uronic acid preparations, Dickson, Otterson, and Link found results about 0.1 to 0.3 per cent below the theoretical, which is very good for this type of method.

Phillips, Goss, and Browne[81] applied the method to a number of plant

[81] *Loc. cit.*

products, with the results given in Table CXXIII, on the basis of material dried at 105° C., except honeydew melon which was dried in vacuo over sulfuric acid.

A micromethod, which is based on the same principles as the macromethod described, has been developed by Burkhart, Baur, and Link,[82] and a semi-micromethod, in which the volume of the liberated carbon dioxide is measured in a gas burette, by Voss and Pfirschke.[83]

DETERMINATION OF *l*-ASCORBIC ACID (VITAMIN C OR CEVITAMIC ACID)

Most of the methods used for the estimation of vitamin C by chemical means are based on its strong reducing power. The principal reagents proposed for this purpose are iodine,[84] methylene blue,[85] the phosphomolybdotungstic acid reagent of Bezssonov,[86] and dichlorophenolindophenol. None of these is strictly specific, but the last named, which has given the best correlation with the standard biologic test, is most generally used. The reaction proceeds according to the equation:

$$\underset{\text{Ascorbic acid}}{C_6H_8O_6} + O{:}C_6H_2Cl_2{:}NC_6H_4ONa \rightarrow \underset{\text{Dehydroascorbic acid}}{C_6H_6O_6} + \underset{\text{(Colorless)}}{HOC_6H_2Cl_2NHC_6H_4ONa}$$

(Blue above *p*H 5.7, Red below *p*H 5.7)

Method of Tillmans, Hirsch, and Hirsch.[87] The dichlorophenolindophenol is dissolved in Sørensen's buffer solution of *p*H 7, which is prepared by mixing 385 ml. of primary phosphate solution (9.078 g. KH_2PO_4 dissolved to 1 liter) with 615 ml. of secondary phosphate solution (11.876 g. $Na_2HPO_4 \cdot 2H_2O$ dissolved to 1 liter). The solution of the reagent is standardized with a 0.01 *N* stock solution of ferrous ammonium sulfate (Mohr's salt) which has been slightly acidified with sulfuric acid and is kept in an atmosphere of nitrogen to prevent oxidation. A measured quantity of this solution is pipetted into a flask, an excess of solid sodium oxalate is added, and the solution of dichlorophenolindophenol is run in rapidly from a burette until the blue color of the dichlorophenolindophenol persists for 1 minute. The titration must be carried out in a dim light because bright light catalyzes reoxidation of the leuco compound. The solution of the dichlorophenolindophenol is diluted so that 10 ml. of it is decolorized by 1 ml. of the standard solution of Mohr's salt. It is kept in a dark bottle and should

[82] *J. Biol. Chem.*, **104**, 171 (1934).
[83] *Ber.*, **70B**, 631 (1937).
[84] Joslyn and Marsh, *Science*, **76**, 82 (1932).
[85] Martini and Bonsignore, *Boll. soc. ital. biol. sper.*, **9**, 388 (1934).
[86] *Compt. rend.*, **182**, 1223 (1926).
[87] *Z. Untersuch. Lebensm.*, **63**, 1 (1932). See also **63**, 241 (1932).

be restandardized every day. A fresh solution should be prepared once a week.

The material to be tested for vitamin C must not come in contact with iron, and the utensils used for comminuting it must be of horn, wood, stainless steel, or the like. The extraction is carried out in an atmosphere of nitrogen. Several volumes of cold or hot water may be used, but best results are obtained by boiling for 10 minutes with 2 to 3 per cent sulfuric acid. The mixture is rapidly cooled, passed through a cloth, and the cloth pressed out. This usually extracts practically all the ascorbic acid, but in some cases the operation must be repeated several times to complete the extraction. The extract is diluted to a definite volume, an aliquot is nearly neutralized with sodium hydroxide, and an excess of solid sodium acetate is added to reduce the acidity to a minimum. The solution is then rapidly titrated with the standard dichlorophenolindophenol. Sometimes the blue color is slowly discharged after the first end point is reached, but this is caused by other substances and should be disregarded. If the extract is so strongly colored that the end point cannot be readily perceived, a centrifuge tube is used for the titration, and a small quantity of nitrobenzene placed in the bottom of it. The dichlorophenolindophenol is soluble in this reagent, while most plant colors are not. The tube is carefully shaken at intervals during the titration. If an emulsion should form it is broken up by short centrifuging. The ascorbic acid value is expressed in milliliters of 0.001 *N* dichlorophenolindophenol on the basis of 10 g. of material extracted.

Method of Emmerie and van Eekelen.[88] These authors remove interfering substances by clarification of the extract and have introduced some further modifications. The material to be analyzed is ground in a mortar with iron-free pure sand and 5 to 10 times the weight of the sample of 3 per cent trichloroacetic acid. The mixture is centrifuged; the supernatant liquid is drawn off, neutralized with precipitated calcium carbonate, and filtered. To remove cysteine and other substances which react with dichlorophenolindophenol, a measured volume of the filtrate is adjusted to *p*H 5 and clarified with 20 per cent mercuric acetate solution, an excess being carefully avoided. The precipitate is quickly removed by centrifuging, and the excess mercury at once precipitated with hydrogen sulfide. This treatment also reduces any reversibly oxidized ascorbic acid to its original state. The liquid is filtered and allowed to stand overnight. The hydrogen sulfide is then blown out by passing nitrogen through the solution for 10 to 30 minutes. Five milliliters of the liquid is pipetted out, 1 ml. of 10 per cent tri-

[88] *Biochem. J.*, 28, 1153 (1934); 30, 25 (1936).

chloroacetic acid is added, and the solution is titrated with dichlorophenolindophenol.

For the determination of ascorbic acid in blood, 10 ml. of the fresh, oxalated blood is mixed in a 100-ml. Erlenmeyer flask with 10 ml. of 10 per cent trichloroacetic acid. After complete precipitation of the protein, 5 ml. of mercuric acetate solution is added and the mixture is well stirred. The trichloroacetic acid is neutralized by shaking with 0.5 g. of calcium carbonate until Congo red paper is colored faintly violet. The mixture is centrifuged, the supernatant liquid drawn off and treated with hydrogen sulfide, and the analysis is completed as described above. Urine to be analyzed is diluted with 5 volumes of water, and 20 ml. of the solution is clarified directly with 10 ml. of mercuric acetate solution; in the case of milk, 25 ml. of sample and 15 ml. of mercuric acetate solution are used.

Method of Bessey and King.[89] This modification of the original method of Tillmans, Hirsch, and Hirsch is the one most generally used in the United States. The reagent is prepared by dissolving 0.1 g. of dichlorophenolindophenol with successive portions of warm water. After cooling, a small amount of phosphate buffer of *p*H 6.8 is added to improve the keeping quality of the reagent. The solution is diluted to 200 ml., filtered, and stored in a dark bottle. The solution must be restandardized daily and made fresh every 5 days. Bessey and King recommend lemon juice for the standardization because its ascorbic acid content can be determined with iodine, without serious error. Five milliliters of fresh, strained lemon juice is titrated with 0.01 *N* iodine solution containing 15 g. potassium iodide per liter, starch being used as indicator. Each milliliter of the iodine solution is equivalent to 0.88 mg. ascorbic acid. Another 5 ml. of the lemon juice is titrated with the dichlorophenolindophenol reagent to a permanent pink, and the ascorbic acid titer is calculated from the two determinations.

To extract the ascorbic acid from the material to be analyzed, 5 to 10 g. of sample is ground to a paste with acid-washed white sand and 25 ml. of 8 per cent acid. Trichloroacetic acid is recommended for animal tissues, and hot acetic acid for plant tissues. The paste is centrifuged and the supernatant liquid decanted. The treatment is repeated with 10 ml. of acid, and once more with 5 ml. The extracts are combined and diluted to 50 ml. A 10-ml. aliquot is diluted with 40 ml. of water or 8 per cent acid, and titrated rapidly with the dichlorophenolindophenol reagent to a faint pink. The end point is reached when rapid fading ceases. With trichloroacetic acid the end point is more difficult to recognize than with acetic acid because of slow fading.

[89] *J. Biol. Chem.*, **103**, 687 (1933).

A blank test is run with the reagents only, and the result is deducted from the titer of the sample. Only water distilled in glass apparatus should be used. The reagent is reduced also by cysteine, glucic acid, pyrogallol, and similar compounds, but the error caused by these substances is only about 2 to 3 per cent for lemon juice. With animal tissues the error may amount to 6 to 8 per cent, but it is not so large as in the biological assay method.

In the case of extracts that are so deeply colored that the color change cannot be readily ascertained, Kirk and Tressler[90] recommend an electrometric method for determining the end point.

The oxidation of ascorbic acid in extracts is hastened by catalase occurring in plant and animal juices, and also by traces of copper, especially if iron is also present. Mack and Tressler[91] have found that more accurate results are obtained by the method of Bessey and King if the action of the catalase is inhibited by extracting with acid of such concentration that the extract has a *p*H of around 1 or lower. Sulfuric or hydrochloric acid may be used, instead of the acids specified by Bessey and King. The effect of the copper is counteracted by adding some 2 per cent metaphosphoric acid to the acid used for extraction, as proposed by Fujita and Iwatake.[92] Mack and Tressler also recommend treating the extract with hydrogen sulfide, as in the method of Emmerie and van Eekelen, but the treatment should not be prolonged beyond thirty minutes, because otherwise the hydrogen sulfide is likely to reduce other substances present in the extract which will then react with the dichlorophenolindophenol. Just before the titration the hydrogen sulfide is removed by a current of carbon dioxide.

Since ascorbic acid has become available in commercial quantities, either from natural sources or in synthetic form, it can be used directly for the standardization of the dichlorophenolindophenol reagent. Glucic acid may also be used as a standard. This is prepared as follows, according to Kertesz.[93] Five milliliters of a 0.5 per cent solution of pure glucose (Bureau of Standards preparation) is placed in a test tube, 0.5 ml. of 0.5 *N* solution of sodium hydroxide is added, and the tube is closed with a Bunsen valve. It is then placed in a water bath heated to 80° C., for exactly 12 minutes. After cooling, 1 ml. of 10 per cent hydrochloric acid is added, making 6.5 ml. in all. This solution is then used to titrate 0.1 to 0.5 ml. of the dichlorophenolindophenol reagent, by means of a microburette. After the pink color disappears,

[90] *Ind. Eng. Chem., Anal. Ed.*, 11, 322 (1939).
[91] *J. Biol. Chem.*, 118, 735 (1937).
[92] *Biochem. Z.*, 277, 293 (1935).
[93] *J. Biol. Chem.*, 104, 483 (1934).

the titrated solution is divided into two parts, and to one of them 1 drop of the glucic acid solution is added, to make sure that the end point had been reached. One milliliter of the glucic acid solution is equivalent to 0.25 mg. of ascorbic acid.

Still another method for standardizing the dichlorophenolindophenol reagent has been introduced by Menaker and Guerrant.[94] The reagent is dissolved in hot water as described by Bessey and King. Fifteen milliliters of the solution is transferred to a 50-ml. Erlenmeyer flask, 0.5 to 1 g. of potassium iodide and 0.5 to 1 ml. of dilute sulfuric acid (1 to 4) are added, and after shaking the iodine set free is titrated with 0.01 *N* thiosulfate solution, starch being used as indicator. One milliliter of 0.01 *N* iodine is equivalent to 0.88 mg. ascorbic acid. A titration of lemon juice with the reagent standardized in this manner gave high values for ascorbic acid, showing that lemon juice contains small amounts of other reducing substances and that the standardization procedure of Bessey and King is subject to error.

Reliability of Ascorbic Acid Determination with Dichlorophenolindophenol. Great care must be exercised in interpreting the results of ascorbic acid determinations by the chemical method. Inconsistencies have been discovered by various investigators, and Kohman and Sanborn[95] have observed that certain plant juices, such as those of peas and beans, contain a complex system of reducing and oxidizing compounds and enzymes. If these juices are allowed to stand for some time in contact with air, alternately lower and higher results are obtained in the titration. This is explained by the reducing effect of glutathione and other substances on dehydroascorbic acid, and by the acceleration of this reduction by an enzyme. The oxidized glutathione in turn is reduced by another enzyme. This interplay of antagonistic reactions interferes with the determination of ascorbic acid, and further studies are necessary to ascertain to what extent these reactions affect the vitamin potency of plant and animal juices.

Determination of Ascorbic Acid in Urine. According to Roe and Hall,[96] normal urines contain substances which cause a plus error in the determination of ascorbic acid by the dichlorophenolindophenol method. Correct results may be obtained by the following procedure. The urine is filtered through Norit decolorizing carbon which treatment oxidizes the ascorbic acid to dehydroascorbic acid. The latter is separated as the 2,4-dinitrophenylosazone which is then reduced by boiling with stannous chloride and hydrochloric acid under pressure. The resulting

[94] *Ind. Eng. Chem., Anal. Ed.*, **10**, 25 (1938).
[95] *Ind. Eng. Chem.*, **29**, 1195 (1937).
[96] *J. Biol. Chem.*, **128**, 329 (1939).

product is distilled with hydrochloric acid, as in the determination of pentoses, and the furfural is determined colorimetrically with aniline acetate (see p. 915). For the details of the method the chemist is referred to the original article.

DETERMINATION OF ACETYLMETHYLCARBINOL, DIACETYL, AND 2,3-BUTYLENE GLYCOL

Acetylmethylcarbinol, identified by Browne[97] as a common constituent of cider vinegar and of fermented cane juice, has since been found in a variety of foodstuffs which have undergone fermentation during the manufacturing process. It is odorless, but in contact with air it is slowly oxidized to diacetyl which has a characteristic odor in great dilution and contributes to the aroma of the foodstuffs, such as butter, bread, and many others. Acetylmethylcarbinol is a by-product of fermentation, formed by molecular rearrangement of acetaldehyde in the presence of yeast:

$$2CH_3—CHO \longrightarrow CH_3—CO—CHOH—CH_3$$

Oxidation converts acetylmethylcarbinol into diacetyl:

$$CH_3—CO—CHOH—CH_3 + O \longrightarrow CH_3—CO—CO—CH_3 + H_2O$$

while reduction changes it to 2,3-butylene glycol:

$$CH_3—CO—CHOH—CH_3 + 2H \longrightarrow CH_3—CHOH—CHOH—CH_3$$

Lemoigne[98] devised a method for the determination of diacetyl, based on the formation of dimethylglyoxime by interaction with hydroxylamine:

$$CH_3—CO—CO—CH_3 + 2NH_2OH \longrightarrow CH_3—CNOH—CNOH—CH_3 + 2H_2O$$

The dimethylglyoxime gives with nickel salts an insoluble precipitate, consisting of red needles and having the formula

$$(CH_3—CNOH—CNO—CH_3)_2Ni$$

Lemoigne's original method has been improved by van Niel.[99] Both diacetyl and acetylmethylcarbinol being volatile, the sum of the two is estimated by first oxidizing the acetylmethylcarbinol to diacetyl, distilling, and determining the total diacetyl as nickel dimethylglyoxime. In another portion of the sample the original diacetyl is determined by

[97] *J. Am. Chem. Soc.*, 25, 31 (1903); 28, 468 (1906).
[98] *Compt. rend.*, 170, 131 (1920).
[99] *Biochem. Z.*, 187, 472 (1927).

distilling in a current of carbon dioxide, to prevent the oxidation of the acetylmethylcarbinol.

Determination of Acetylmethylcarbinol Plus Diacetyl. A 100- to 200-g. sample is dissolved or suspended in water in a distilling flask, an excess of ferric chloride solution (50 ml. of a 30 per cent solution) is added to oxidize the acetylmethylcarbinol, and the mixture is distilled slowly into a receiver containing, for each 100 mg. diacetyl, 2 ml. of a 20 per cent solution of hydroxylamine hydrochloride, 3 to 5 ml. of a 20 per cent solution of sodium acetate, and 1 to 2 ml. of a 10 per cent solution of nickel chloride (anhydrous). When three-fifths of the solution has distilled over, the receiving flask is tightly stoppered and placed in a water bath at 80° C. for at least an hour. It is then allowed to cool and to stand for 2 days or longer at room temperature, to complete crystallization. The precipitate is then filtered through a Gooch or sintered glass crucible, washed with water, dried at 110° C., and weighed. If fatty substances have distilled over, the washing with water must first be followed by drying, and washing with petroleum ether, before drying to constant weight. The weight of the precipitate, multiplied by 0.596, gives the sum of acetylmethylcarbinol and diacetyl, as diacetyl.

Determination of Acetylmethylcarbinol. Another portion of the sample is distilled, without the addition of ferric salt, in a current of carbon dioxide, in the same manner as described above. The diacetyl found is deducted from the total diacetyl obtained in the first determination, and the difference is multiplied by 1.023 to convert it into acetylmethylcarbinol.

In the analysis of butter, bread, and similar products it is preferable to conduct the distillations in a current of steam.[100] A special apparatus for this purpose has been described by Visser't Hooft and de Leeuw.[101] According to these authors only 80 per cent of pure acetylmethylcarbinol is recovered upon distillation in the presence of ferric chloride. In similar experiments with van Niel's method, Stahly and Werkman[102] obtained 84 per cent, and conclude from this that the result calculated as shown above must be divided by 0.84. The chemist should therefore always run parallel distillations with pure diacetyl and pure acetylmethylcarbinol under his own experimental conditions and apply a correction factor based on the results of these tests.

If the quantity of nickel dimethylglyoxime is so small that it cannot

[100] Michaelian and Hammer, *Iowa Agr. Expt. Station, Research Bull.* 179, p. 204, 1935.

[101] *Cereal Chem.*, 12, 213 (1935).

[102] *Iowa State Coll. J. Sci.*, 10, 205 (1936).

be weighed accurately, it can be determined by dissolving it in chloroform and comparing colorimetrically with standards prepared from known quantities of diacetyl.[103]

In experiments with pure diacetyl Schmalfuss and Rethorn[104] found that it can be recovered with a loss of only 0.2 per cent by the following procedure. A mixture of 50 ml. water, 2 ml. of a 20 per cent solution of hydroxylamine hydrochloride, and 3 ml. of 10 per cent nickel chloride solution is prepared in an Erlenmeyer flask and cooled to 0° C. The diacetyl, previously cooled to —10° C., is added with shaking at 0° C. Then 2 to 3 times the calculated amount of 20 per cent ammonia, also cooled to 0° C., is added, and the flask stoppered and shaken. It is allowed to stand at room temperature for 10 minutes, placed in an unheated water bath, the water heated to boiling, and the flask kept in the boiling-water bath for 1½ hours. It is again cooled to 0° C. The precipitate is filtered through a sintered glass funnel, being transferred and washed with about 100 ml. of ice water, dried to constant weight at 110 to 120° C., cooled in a desiccator over phosphorus pentoxide, and weighed after 2 hours.

Determination of 2,3-Butylene Glycol. The method of van Niel described above may also be used for the determination of 2,3-butylene glycol, which frequently occurs in fermentation products. According to Moureu and Dodé[105] the butylene glycol is oxidized to acetylmethylcarbinol by the addition of an excess of bromine water in a glass tube which is then sealed with the blowpipe and heated for 3 minutes in a boiling-water bath. After the tube has been allowed to cool in the dark, the seal is broken, the excess bromine removed by careful addition of 20 per cent sodium sulfite solution, and the acetylmethylcarbinol determined as nickel dimethylglyoxime by oxidation with ferric salt and distillation according to van Niel's method. Only 75.6 per cent of the butylene glycol is transformed into acetylmethylcarbinol by the above procedure, and the weight of nickel dimethylgloxime must therefore be multiplied by 0.824, instead of the theoretical factor 0.624, to convert it to 2,3-butylene glycol.

In a method devised by Brockmann and Werkman[106] the 2,3-butylene glycol is distilled from the sample in a current of steam. An aliquot of the distillate is placed in an Erlenmeyer flask connected with a reflux condenser, potassium periodate and sulfuric acid are added to oxidize

[103] Barnicoat, *Analyst*, 60, 653 (1935).
[104] *Z. Untersuch. Lebensm.*, 70, 233 (1935).
[105] *Bull. assoc. chim. sucr. dist.*, 51, 247 (1934).
[106] *Ind. Eng. Chem., Anal. Ed.*, 5, 206 (1933); see also Stahly and Werkman, *Iowa State Coll. J. Sci.*, 10, 205 (1936).

the 2,3-butylene glycol to acetaldehyde, the liquid is heated to boiling, and the acetaldehyde is driven by means of a current of air into an absorption tower containing a known volume of standard hydroxylamine hydrochloride solution. The formation of the aldoxime liberates hydrochloric acid which is titrated with standard sodium hydroxide solution. Any acetylmethylcarbinol present in the sample reacts as 2 moles of acetaldehyde; it must therefore be determined in a separate portion of the sample by van Niel's method, and a correction applied to the apparent 2,3-butylene glycol found. For the details of the method the original should be consulted.

A colorimetric procedure for the determination of diacetyl, and thus indirectly of acetylmethylcarbinol and of butylene glycol, has been proposed by Pien, Baisse, and Martin.[107] It is based on the formation of a yellow quinoxaline compound from diacetyl and 4-methylorthophenylenediamine.

Acetylmethylcarbinol causes an error in reducing-sugar determinations because it reduces alkaline copper or ferricyanide solutions. In the method of Munson and Walker, 1 mg. produces 3 mg. of cuprous oxide; in that of Shaffer and Hartmann, 2.3 mg.[108]

DETERMINATION OF GALACTOSE OR GALACTAN

Tollens[109] and his coworkers have developed a method for estimating galactose and its higher condensation product galactan $(C_6H_{10}O_5)_n$, which is based upon a determination of the mucic acid formed by oxidation of the substance with nitric acid. The oxidation of galactose to mucic acid according to theory proceeds as follows:

$$\underset{\text{Galactose (180)}}{C_6H_{12}O_6} + 2HNO_3 \longrightarrow \underset{\text{Mucic acid (210)}}{C_6H_{10}O_8} + 2H_2O + 2NO$$

One hundred parts of galactose thus equal 116.66 parts of mucic acid. In actual experiment only about 75 per cent of the weight of galactose is obtained as mucic acid. This yield, however, is fairly constant for the given conditions of analysis, so that the weight of mucic acid multiplied by $1\frac{1}{3}$ gives the weight of galactose.

The method of Tollens as employed by the Association of Official Agricultural Chemists[110] is as follows:

[107] *Lait*, **16**, 119, 243 (1936).

[108] Langlykke and Peterson, *Ind. Eng. Chem., Anal. Ed.*, **9**, 163 (1937). These authors describe also a method for determining acetylmethylcarbinol by oxidation with sodium hypoiodite.

[109] *Ann.*, **227**, 223 (1885); **232**, 187 (1886).

[110] "Methods of Analysis, A. O. A. C.," 5th ed., pp. 362–363, 1940.

Extract a convenient quantity of the sample, representing 2.5–3 g. of the dry material, on a hardened filter with 5 successive portions of 10 ml. of ether; place the extracted residue in a beaker, about 5.5 cm. in diameter and 7 cm. deep; add 60 ml. of nitric acid (sp. gr. 1.15); and evaporate on a steam bath to a volume of 20 ml. Let stand 24 hours, then add 10 ml. of water and allow to stand another 24 hours. Pass through a filter and wash the impure mucic acid crystals with 30 ml. of water to remove as much of the nitric acid as possible, and return filter and contents to the original beaker. Add 30 ml. of $(NH_4)_2CO_3$ solution (consisting of 1 part $(NH_4)_2CO_3$, 19 parts water, and 1 part of NH_4OH) and heat the mixture in a water bath, at 80°, for 15 minutes, with constant stirring. The $(NH_4)_2CO_3$ combines with the mucic acid, forming soluble NH_4 mucate. Wash the filter paper and contents several times with hot water by decantation, passing the washings through a filter paper, to which finally transfer the residue, and wash thoroughly. Evaporate the filtrate to dryness on a water bath, avoiding unnecessary heating which causes decomposition; add 5 ml. of nitric acid (sp. gr. 1.15); stir the mixture thoroughly; and allow to stand for 30 minutes. Collect the precipitated mucic acid on a weighed Gooch crucible or other filter; wash with 10–15 ml. of water, then with 60 ml. of 95 per cent alcohol, and then a number of times with ether; dry at the temperature of boiling water for 3 hours; and weigh. Multiply the weight of the mucic acid by 1.33 to convert to galactose, and by 1.20 to convert to galactan.

The method of Tollens has been used considerably by Schulze and Steiger[111] for determining galactan groups in different plants of the Leguminosæ and also by Bauer[112] for estimating galactose and lactose in the urine.

The presence of large amounts of foreign organic matter hinders the precipitation of mucic acid, and in case of only small amounts of the latter may prevent its separation entirely. The tendency of the method, therefore, is to give too low rather than too high results.

Method of van der Haar. The method of Tollens has been found by various investigators to be unreliable, especially when used on materials containing galactan in mixture with other carbohydrates. Van der Haar[113] studied this subject further and devised a method which gives much better results, as confirmed by Wise and Peterson.[114] This procedure, with slight modifications introduced by the last-named authors, is carried out as follows:

In the case of galactose alone, not more than 1 g. of the dry substance is used for analysis; if other sugars are present, enough dry pure sucrose

[111] *Landw. Vers. Stat.*, 36, 11, 438, 465 (1889).
[112] *Z. physiol. Chem.*, 51, 159 (1907).
[113] *Biochem. Z.*, 81, 263 (1917).
[114] *Ind. Eng. Chem.*, 22, 362 (1930).

is added to bring the dry weight to exactly 1000 mg. The dry material is placed in a beaker, 6 cm. wide by 12 cm. high, and 60 ml. of nitric acid of sp. gr. 1.15 at 15° C. (25 per cent nitric acid) is added.

Products containing galactan are first hydrolyzed by heating with sulfuric acid of 2 to 5 per cent strength for several hours at 105° to 110° C. After cooling, barium hydroxide solution is run in under constant stirring until only a slight acidity remains. The neutralization is completed by the addition of pure barium carbonate. The mixture is heated to 80° C. and allowed to stand overnight, after which it is filtered, and the filtrate made up to a definite volume. Aliquots are evaporated in vacuo to such a concentration that 30 ml. corresponds to about 0.6 to 0.9 g. of the original polysaccharide. If the concentration is less than that corresponding to 0.9 g., enough dry sucrose is added to bring the total calculated sugar content to 1000 mg. The 30 ml. solution is mixed, in the 6 by 12 cm. beaker, with 30 ml. of nitric acid of sp. gr. 1.315 at 15° C. (50 per cent nitric acid), so that the final nitric acid concentration is the same as that given above for the analysis of dry materials.

In all cases the beaker with 60 ml. of total liquid is reweighed, and placed, in an inclined position, in a boiling-water bath, being shaken at frequent intervals, until the weight of the contents is reduced to 20 ± 0.1 g. If the evaporation is carried beyond this point, the oxidation may proceed further than the mucic acid stage. After cooling, 500 mg. of pure mucic acid is added to promote crystallization, which is allowed to proceed for 48 hours in a bath kept at 15 ± 0.5° C., with occasional shaking. The Gooch crucible to be used for the collection of the mucic acid is first washed with nitric acid, then with water, dried, ignited, and weighed. The mucic acid crystals are filtered off on the Gooch crucible and washed with 8 to 10 portions of 10 ml. each of a saturated mucic acid solution which has been standing for at least 48 hours at 15° C. and has been filtered before use. The final washing is made with 5 ml. of water. The crucible is dried for several hours, but not overnight, at 100° C., and weighed. From the total weight of precipitate found the 500 mg. mucic acid originally added is deducted, and the weight of galactose corresponding to the difference is found from the tables of van der Haar (Appendix, Tables 33 and 34). The first of these tables is used if only galactose is present, the second if the weight of the sugars present has been increased to 1000 mg. by the addition of sucrose. To find galactan, the weight of galactose is multiplied by 0.846.

In the analysis of milk sugar, to which enough sucrose had been added to increase the total sugars to 1000 mg., van der Haar obtained

results closely agreeing with the theoretical galactose content, but the weight of mucic acid obtained in duplicate determinations varied as much as 8 mg.

Galacturonic acid also yields mucic acid upon treatment with nitric acid, but the ratios between the two, for varying amounts of galacturonic acid, have not been determined. Theoretically, 1.08 parts galacturonic acid should give the same quantity of mucic acid as 1 part of galactose.

DETERMINATION OF CELLULOSE, LIGNIN, AND THE METHOXYL GROUP

In the analysis of plant materials the chemist is frequently called upon to determine not only pentosans, galactan, and other hemicelluloses, but also the less-soluble constituents of the plant fiber, cellulose and lignin.

Determination of Cellulose. The simplest procedure for the proximate estimation of cellulose is the well-known " crude fiber " method of Henneberg,[115] commonly employed in the analysis of feed stuffs. The crude fiber consists principally of cellulose, but the dilute acid and alkali used remove the lignin, pentosans, and other impurities only partly. To purify the cellulose more completely, Schulze,[116] in 1857, proposed digestion with dilute nitric acid and potassium chlorate, and a number of other procedures have been devised for the purpose. The most widely used method is that of Cross and Bevan.[117]

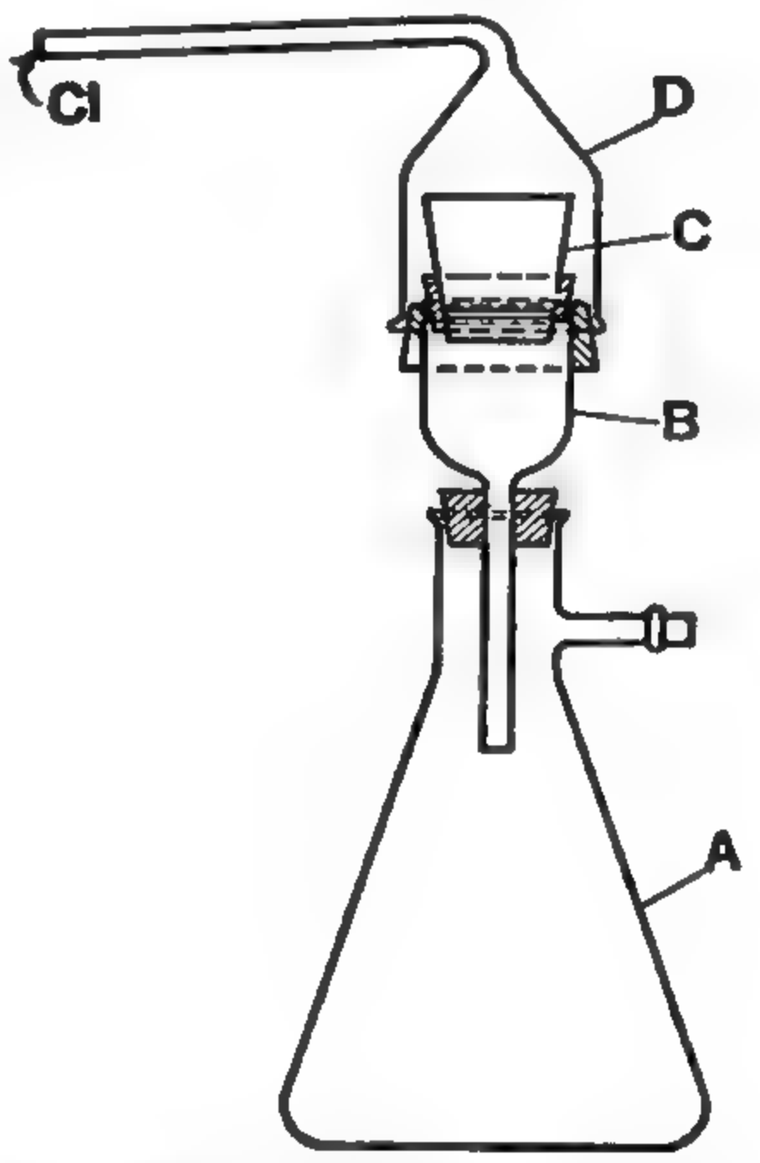


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FIG. 295. Apparatus for determining cellulose.

Method of Cross and Bevan. The original method of these authors has been variously modified. According to the modification of Phillips[118] it is carried out as follows. The apparatus used, designed by Sieber and Walter,[119] is shown in Fig. 295. *A* is a 1-liter suction flask, to which the Jena fritted glass crucible *C* (porosity 2–3, 60-ml. capacity) is attached by means of adapter *B*. A second adapter *D* is fitted over *B*, making an air-tight

[115] *Weender Beiträge*, No. 2, 48 (1864).
[116] *Chem. Zentr.*, 1857, 321.
[117] *J. Chem. Soc.*, 55, 199 (1889).
[118] *J. Assoc. Official Agr. Chem.*, 15, 118 (1932).
[119] *Papier-Fabr.*, 11, 1179 (1913).

connection. Washed chlorine gas is aspirated through the apparatus by means of a water vacuum pump made of glass.

The material to be analyzed is coarsely ground and dried at 105° C., and a weighed sample, about 5 g., is extracted for 6 hours in a Soxhlet extractor with a mixture of 32 parts of alcohol and 68 parts of benzene. It is then dried by suction, washed with hot water, and dried again at 105° C., and the loss in weight is determined.

Two 1-gram samples of the extracted and dried material are weighed out in fritted glass crucibles, preferably from a weighing tube. One of the crucibles is placed in *B* and washed with water, and adapter *D* is fitted over the crucible as shown in the illustration. The vacuum pump is set in action, and a slow current of chlorine, washed with water, is passed through for 5 minutes, at the rate of 1 bubble per second. The adapter *D* is then removed, and the material in the crucible is washed first with a dilute sulfurous acid solution and then thoroughly with water. The crucible is placed in a 250-ml. beaker which is filled with 2 per cent sodium sulfite solution to within ½ inch of the top of the crucible. The beaker is then placed on the steam bath, and the contents are digested for 30 minutes. During this time the other crucible is treated exactly like the first one, and also placed on the steam bath for a 30-minute digestion. Each crucible is then placed in turn in the adapter *B*, filtered under suction, washed with water, and again chlorinated and digested with sodium sulfite solution, as above described. This procedure is repeated until all the lignin has been removed, as shown by the disappearance of the reddish violet coloration which lignin gives with sodium sulfite solution. The cellulose in the crucibles is finally bleached by adding 20 ml. of 0.1 per cent potassium permanganate solution, allowing to stand at room temperature for 20 minutes, and rendering colorless by washing with dilute sulfurous acid. It is then washed with a very dilute solution of ammonia, hot water, 95 per cent alcohol, and ether, dried at 105° C., and weighed. A portion of the cellulose is weighed into an ignited and weighed porcelain crucible and ashed, and the ash is weighed. The percentage of ash-free cellulose in the original dry, unextracted material is then calculated, and the result is reported as " Cross and Bevan " cellulose.

The cellulose thus obtained usually yields some furfural when distilled with 12 per cent hydrochloric acid according to Tollens's procedure. It is not known whether this furfural is due to the presence of pentosans, oxycellulose, or some other substance. If desired, the furfural may be determined quantitatively and reported as " per cent furfural in Cross and Bevan cellulose."

A rapid method for the determination of cellulose, based on its in-

solubility in hot monoethanolamine, has been devised by Reid, Nelson, and Aronovsky,[120] especially for the analysis of farm wastes. The results compare favorably with those of the Cross and Bevan method, the pentosan content of the cellulose obtained being usually a little higher, and the lignin content a little lower.

For other methods of cellulose determination the chemist is referred to the special works on this subject.

Determination of Lignin. This substance, as its name implies, is a characteristic constituent of woody materials in which it forms a complex with cellulose, pentosans, and other hemicelluloses. When isolated it forms a brown, amorphous powder, soluble in alkalies but insoluble in water and in acids. Although its chemical nature has not been fully elucidated, it is probably of carbohydrate origin and is always found associated with cellulose. The lignin of wood appears to take the place of the pectin (see p. 1180) occurring in the softer skeletal parts of plants, and there may be a generic relation between the two, both of them containing methoxyl groups and both being acidic in nature. Aromatic compounds have been obtained by alkali fusion of lignin, and some investigators believe that lignin is essentially a polymer of coniferyl aldehyde, but this view has not been accepted by others.

Lignin may be separated from the accompanying cellulose by treatment with alkali, but for quantitative purposes it is preferable to dissolve the cellulose in the cold with strong (66 to 80 per cent) sulfuric acid,[121] or with fuming hydrochloric acid.[122] Goss and Phillips[123] have made a comparative study of several methods based on this principle, and particularly of the pretreatment necessary to remove interfering substances, such as fats and waxes, and also soluble carbohydrates which form humuslike substances insoluble in strong acids.

Method of Goss and Phillips. The apparatus used in this method is shown in Fig. 296, and the procedure is described by the authors as follows.

Preparation of Sample. — The plant material is ground in a mill fine enough to pass an 80-mesh sieve, and dried at 105° C. A weighed sample (5–10 g.) is extracted for 30 hours in a Soxhlet apparatus with a mixture of 32 parts by weight of alcohol and 68 parts of benzene. It is then dried in an oven to remove the alcohol and benzene, and placed

[120] *Ind. Eng. Chem., Anal. Ed.*, 12, 255 (1940).

[121] Flechsig, *Z. physiol. Chem.*, 7, 523 (1883); Klason, *Ber. Ver. Papier und Zellstoffchem.*, 1908, p. 52.

[122] Willstätter and Zechmeister, *Ber.*, 46, 2401 (1913).

[123] *J. Assoc. Official Agr. Chem.*, 15, 118 (1932); 17, 277 (1934); 18, 386 (1935); 19, 341, 350 (1936).

in a flask of suitable size. For each gram of sample, 150 ml. of distilled water is added, and the mixture is boiled under reflux for 3 hours. It is then filtered hot, preferably through a weighed Jena sintered glass crucible. The extracted material is transferred to a flask and boiled under reflux for 3 hours with 1 per cent hydrochloric acid, in the proportion of 150 ml. to each gram of sample. The mixture is filtered hot through the glass crucible used in the previous operation, washed with distilled water until free of acid, dried at 105° C., and weighed. The percentage loss due to the successive extractions with alcohol-benzene, water, and dilute acid is calculated.

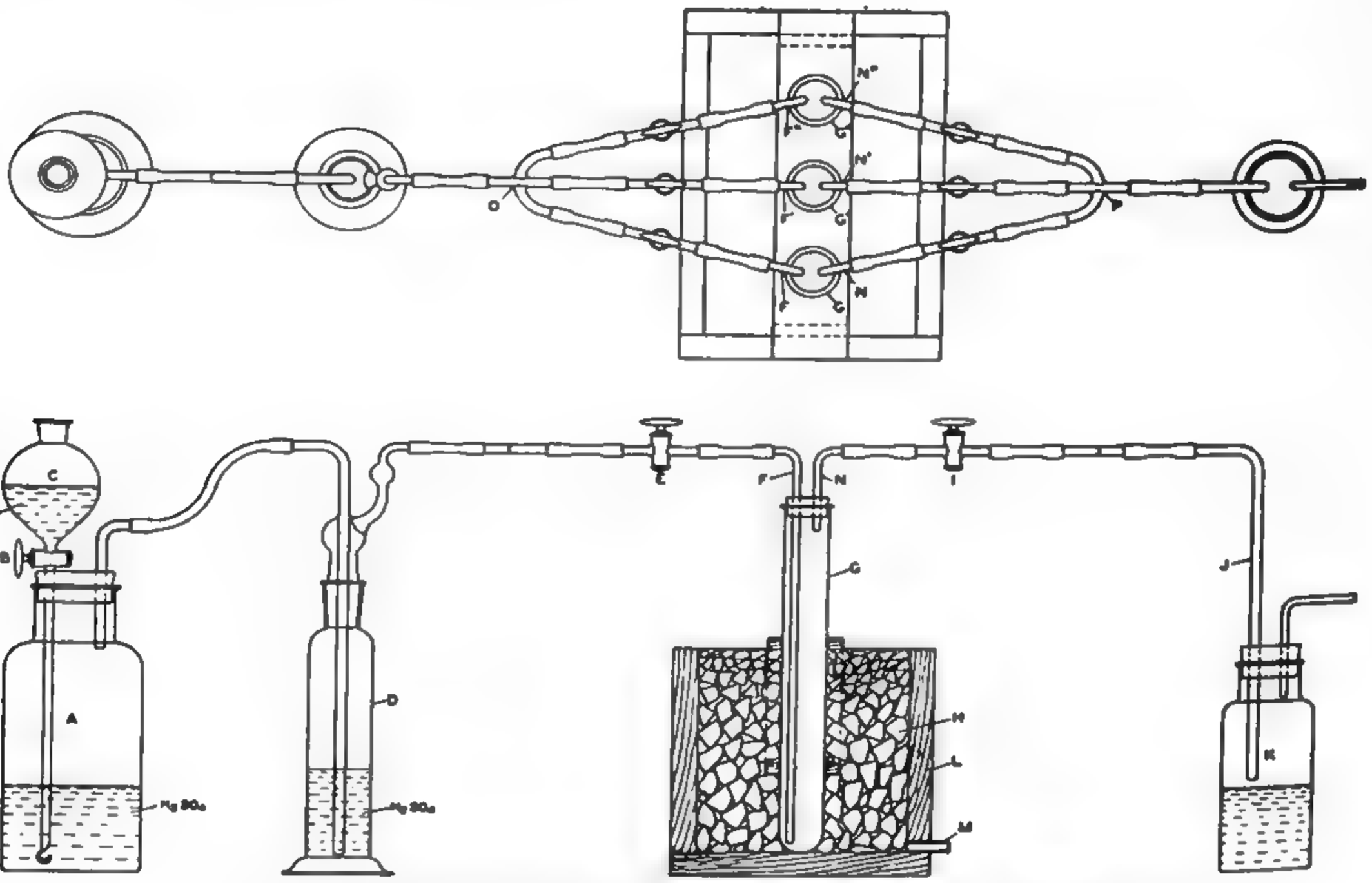

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FIG. 296. Apparatus for determining lignin.

Determination. — Three 1-g. samples of the extracted and dried material are weighed out from a weighing bottle and placed in the three large test tubes, *G*, *G′*, and *G″* (Pyrex, 12 inches long and 1.5 inches in inside diameter), and 20 ml. of fuming hydrochloric acid (sp. gr. 1.212–1.223 at 15° C.) is added to each tube, care being taken to wash down with this acid any particles clinging to the sides. When all the material is wetted, another, 30-ml. portion of the fuming acid is added, followed by 3 drops of capryl alcohol to reduce the foaming during the subsequent passage of hydrogen chloride gas through the reaction mixture. The three test tubes, *G*, *G′*, and *G″*, are placed in a wooden box (*L*) and surrounded with crushed ice. The inlet tubes *F*, *F″*, and *F‴*

are lubricated with a drop of glycerol, the purpose being to allow them to move freely through the holes in the rubber stoppers. Hydrogen chloride gas is generated by allowing concentrated hydrochloric acid to run from the dropping funnel *C* into concentrated sulfuric acid in *A*, and is dried by passing it through wash bottle *D* containing concentrated sulfuric acid. The dried gas is led into the reaction mixture through the inlet tubes, which reach nearly to the bottom of the test tubes. The gas flow is continued for 2 hours, the speed being regulated by the glass stopcocks shown in the top view. At first a rather slow stream of gas is passed in, but during the last 15 minutes the flow is fairly rapid. The excess gas is absorbed in water in bottle *K*.

At the end of the reaction period the flow of gas is stopped, and the inlet tubes *F*, *F'*, and *F''*, as well as the outlet tubes *N*, *N'*, and *N''*, are disconnected from *O* and *P*. The tubes *F*, *F'*, and *F''* are pulled up just above the surface of the reaction mixture and are closed by means of short pieces of rubber tubing plugged with short pieces of glass rod. The three outlet tubes are similarly closed off. The test tubes containing the reaction mixtures, with the inlet and outlet tubes attached, are placed in a cold room or icebox at 8 to 10° C., and allowed to remain there for 24 hours. The contents are then transferred to three 1-liter Erlenmeyer flasks, care being taken to remove any material adhering either on the inside or outside of *F*, *F'*, and *F''*. Enough distilled water is added in each flask to make a total volume of 500 ml.; the flasks are connected with reflux condensers and boiled for 1 hour.

Three Gooch crucibles are prepared in the usual manner, dried at 105° C., and weighed. One of the weighed crucibles (*A*) is ignited over a Bunsen burner, cooled in a desiccator, and reweighed. The contents of the three flasks are allowed to cool to room temperature and filtered through the weighed Gooch crucibles. The precipitates collected in the crucibles are washed with hot water and dried at 105° C., and the crucibles are weighed in a weighing bottle. The crude lignin in crucible *A* is ignited over a Bunsen flame, and the weight of the ash is determined. One of the other two crucibles is placed in a Kjeldahl flask with a wide neck, and the percentage of nitrogen in the crude lignin is determined by the Kjeldahl-Gunning-Arnold method.[124] If it is desired to determine the percentage of methoxyl in the lignin (see p. 945), the precipitate from one of the flasks is collected in a Jena sintered glass crucible, previously dried at 105° C., and weighed. The weight of the lignin in the sample is computed as follows: Weight of lignin equals weight of crude lignin, minus weight of ash, minus weight of crude pro-

[124] "Methods of Analysis, A. O. A. C.," 5th ed., p. 26, 1940.

tein (N × 6.25).[125] The percentage of lignin in the original dry unextracted material is then calculated.

In comparative determinations by this method, and by the methods of Ritter, Seborg, and Mitchell,[126] of Peterson, Walde, and Hixon,[127] and of Schwalbe,[128] in all of which 72 per cent sulfuric acid is employed, Phillips and Goss found that the hydrochloric acid method gives the lowest results. But the lignin obtained generally contains more methoxyl and is therefore considered to be of higher purity. Whatever method is used, the material must first be extracted as described to remove accompanying impurities.

Ritter and Barbour[129] have found that if catechol tannins accompany the lignin they must be removed by extraction with 95 per cent alcohol prior to the treatment with benzene-alcohol mixture. For lignin determination in sugar beets Nowotnowna and Wiadrowska[130] recommend extraction with ether, 96 per cent alcohol, and a mixture of benzene and ether.

In the method of Popov[131] the material to be analyzed is first extracted with dilute hydrochloric acid, washed, and dried. About 1 g. of the residue is digested for 10 hours with 20 to 30 ml. of a solution containing 40 g. zinc chloride, 100 ml. 37 per cent hydrochloric acid, and 5 to 10 ml. of water. The insoluble portion is filtered off with suction, washed with two portions each of 5 ml. of the solvent, then with cold and finally with hot water until free from hydrochloric acid. It is then dried and weighed, and the pure lignin is found from the loss in weight upon ignition. The results checked with those obtained by dissolving the cellulose with 41 per cent hydrochloric acid.

Klatt[132] has proposed the use of hydrofluoric instead of sulfuric or hydrochloric acid to dissolve the cellulose, while Lemmel[133] has reported that lignin can be separated quantitatively from cellulose by extracting the lignin with ethyl acetoacetate in the presence of hydrochloric acid. These methods require further study.

[125] Phillips has found that the nitrogen in the lignin does not always represent protein (*J. Assoc. Official Agr. Chem.*, **22**, 422 (1939). It is safer to report the ash-free lignin and the percentage of nitrogen it contains.

[126] *Ind. Eng. Chem., Anal. Ed.*, **4**, 202 (1932).

[127] *Ind. Eng. Chem., Anal. Ed.*, **4**, 216 (1932).

[128] *Papier-Fabr.*, **23**, 174 (1925).

[129] *Ind. Eng. Chem., Anal. Ed.*, **7**, 238 (1935).

[130] *Gaz. Cukrownicza*, **76**, 270 (1935).

[131] *Z. Tierernähr. Futtermittelk.*, **1**, 245 (1938).

[132] *Angew. Chem.*, **48**, 112 (1935).

[133] *Anales soc. españ. fis. quím.*, **33**, 389 (1935).

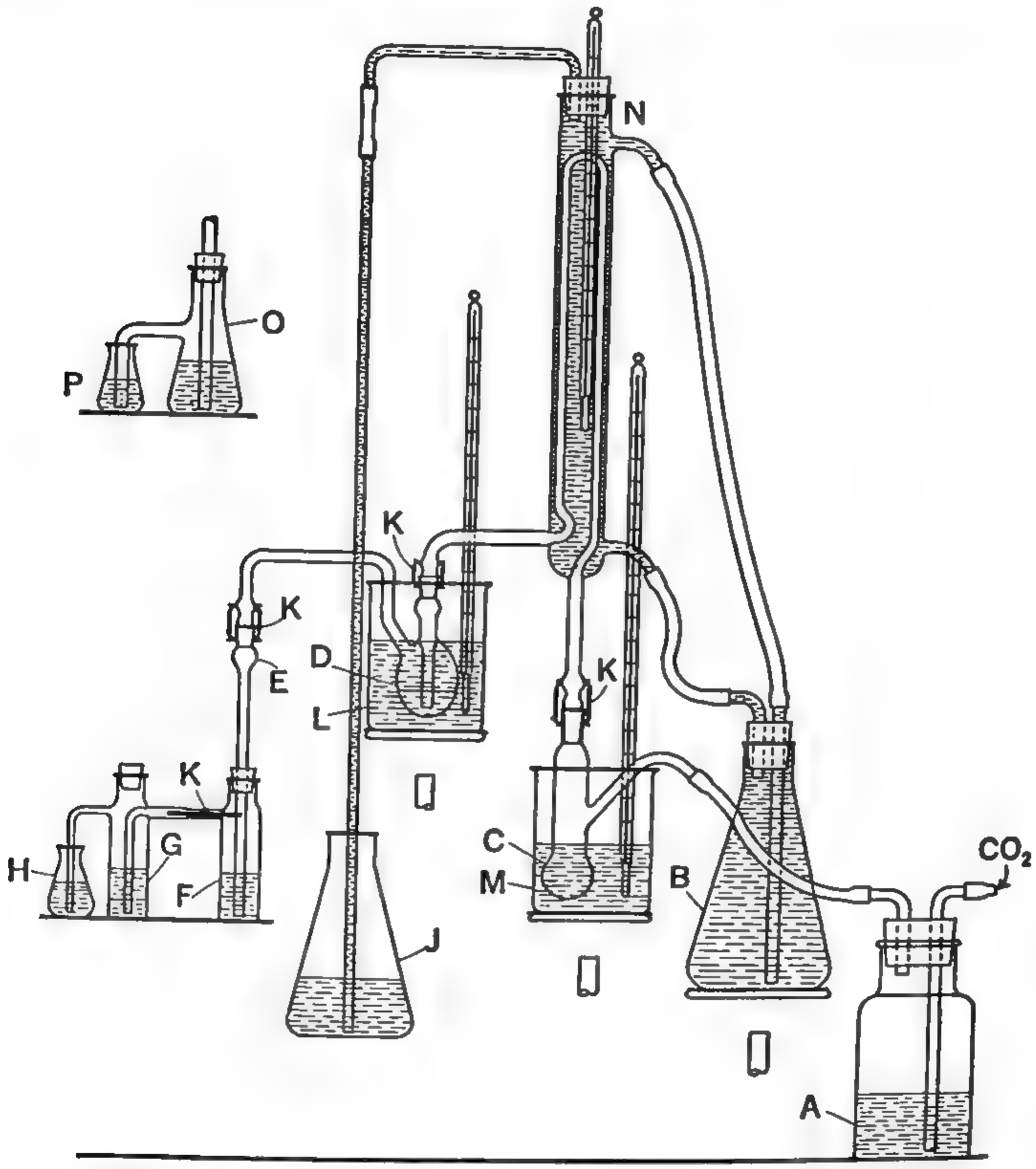
Determination of Methoxyl. The methoxyl group, CH_3O, that is the hydroxyl group in which the hydrogen is replaced by methyl, is found in many substances occurring in nature, among them lignin and pectin. The determination of methoxyl serves to check the purity of the lignin or pectin obtained from various sources, and to study their degradation products. Zeisel[134] devised a method for this purpose in 1885. It is based on the fact that, when a compound containing an alkoxyl group, CH_3O, C_2H_5O, etc., is heated with hydrogen iodide, the alkyl group is replaced by hydrogen and combines with the iodine to form alkyl iodide. The iodine in the latter is then determined by precipitating with silver nitrate in alcoholic solution and weighing as silver iodide. If only methoxyl is to be determined, and there is a possibility of ethoxyl being present also, the two may be separated, according to Phillips,[135] by absorbing the iodides in pyridine which takes up the methyl iodide quantitatively, with the formation of a pyridinium salt, while ethyl iodide reacts very sluggishly. The excess pyridine is then evaporated off, and the iodine in the pyridinium salt is determined as silver iodide.

Zeisel Method, as Modified by Phillips. The apparatus used in this method is shown in Fig. 297. A sample of the material, which has previously been dried at 105° C., is weighed out from a weighing bottle into reaction flask *C*. Ten milliliters of hydriodic acid and 3 ml. of phenol, U.S.P. grade, and a few pieces of porous tile are added to the sample, and the reaction flask is connected by a ground-glass joint to the inner tube of condenser *N*. Water from flask *B*, heated to 50–60° C., is circulated through the outer tube of the condenser, the overflow being caught in flask *J*. The other end of the inner tube of the condenser is connected through a ground-glass joint with the scrubbing flask *D*. This is one-third filled with a thin suspension of red phosphorus, which has been purified by digesting it on the water bath with dilute ammonia for ½ hour, filtering, and washing with distilled water; it is kept in the wet state in a well-stoppered flask. The phosphorus suspension absorbs any free iodine which may have come over with the methyl iodide. *D* is immersed in a water bath *L*, which is heated to 50–60° C. It is connected by a ground-glass joint with the absorption flasks *F*, *G*, and *H*. *F* and *G* are connected by a ground-glass joint, and both are half filled with chemically pure pyridine. *H* is half filled with water, to absorb the pyridine vapors. All ground-glass joints are held fast by means of bronze springs *K*.

After the apparatus has been connected, a current of carbon dioxide,

[134] *Monatsh.*, **6**, 989 (1885); **7**, 406 (1886).
[135] *J. Assoc. Official Agr. Chem.*, **15**, 118 (1932).

washed with concentrated sulfuric acid in bottle *A*, is passed through at the rate of 1 bubble per second. The reaction flask *C* is then inserted in the glycerin bath *M*, which is heated to 130–140° C., and the heating is continued for an hour and a half. During the last 15 minutes a fairly rapid stream of carbon dioxide is passed through in order to sweep all the methyl iodide into the absorption flasks. These are then discon-



(*Reproduced with permission from J. Assoc. Official Agr. Chem., 15, 122.*)

FIG. 297. Apparatus for determination of methoxyl.

nected, and after their contents have been carefully transferred to a 400-ml. beaker they are thoroughly washed out by means of a stream of distilled water from a wash bottle. The beaker is then placed on a water bath, and the solution is evaporated to dryness. The residue is dissolved in distilled water, and if the solution is not clear, it is filtered through a small filter paper. Ten drops of nitric acid is added and then

an excess of 5 per cent aqueous silver nitrate solution. The mixture is brought to a boil, set aside, and allowed to cool to room temperature. The silver iodide is filtered in a weighed Gooch crucible which has been dried at 105° C. The crucible and its contents are dried at the same temperature and weighed. The weight of the silver iodide, multiplied by the factor 0.1321, gives the weight of methoxyl in the sample. From this result the percentage of methoxyl in the sample can be calculated.

The iodine in the pyridinium methyl iodide may also be determined by the volumetric Volhard method, as described by Hewitt and Jones.[136]

If it is desired to absorb the methyl iodide in alcoholic silver nitrate solution, instead of pyridine, the absorption flasks *F*, *G*, and *H* are replaced by similar bottles *O* and *P*, half filled with a solution of 20 g. of silver nitrate in 50 ml. of water diluted with 450 ml. of 95 per cent alcohol which has been redistilled over sodium hydroxide. After the distillation the solution and precipitate in *O* and *P* are carefully washed into a beaker, 10 drops of concentrated nitric acid is added, and the mixture is digested on a steam bath for ½ hour. This procedure breaks up the double salt $AgI,2AgNO_3$ into AgI. The precipitate is collected in a weighed Gooch crucible as described above, dried at 105° C., and weighed. If this method is used, the hydriodic acid employed must be free of sulfur. By a comparison of this method with the pyridine absorption method Phillips found that lignin from oat hulls contains only methoxyl groups.

Method of von Fellenberg. Myers and Baker[137] have found that the methoxyl in pectin can be determined more simply and quite as accurately by the hydrolysis method of von Fellenberg.[138] A 1-gram sample of the dried pectin is dissolved in carbon-dioxide-free water and diluted to 200 ml. in a flask, care being taken to protect the solution from air. The acidity of the solution is exactly neutralized with standard, carbon-dioxide-free sodium hydroxide, using phenolphthalein indicator. An excess of 20 ml. of 0.5 *N* sodium hydroxide is then added, and the solution allowed to stand 2 hours at room temperature. The excess alkali is determined by titration with 0.5 *N* sulfuric acid. The sodium hydroxide equivalent to the methoxyl is then calculated, and expressed as per cent CH_3O.

Too large an excess of alkali or too high a temperature must be avoided, to prevent the splitting off of acetyl groups or degradation of the pectin. If the method is carefully conducted the results check fairly well with those of the methyl iodide method.

[136] *J. Chem. Soc.*, 115, 193 (1919).
[137] *Delaware Agr. Expt. Station Bull.* 187, p. 11, 1934.
[138] *Mitt. Lebensm. Hyg.*, 5, 225 (1914).

FERMENTATION METHODS FOR DETERMINING SUGARS

A method for estimating sugars has been described (p. 485) which is based upon the change in polarization which the solution undergoes after fermenting with yeast.

The fermentation methods for determining sugars are more usually carried out by weighing or measuring the carbon dioxide which is evolved. The theoretical yield of carbon dioxide from glucose, according to the equation $C_6H_{12}O_6 = 2\,C_2H_5OH + 2\,CO_2$, is 48.88 per cent. In actual experiments only about 45 per cent of carbon dioxide is obtained, this figure varying, however, by several per cent according to the variety of yeast, influence of non-sugars, and other conditions. The weight of carbon dioxide obtained during a normal fermentation multiplied by the factor 2.2 will give the approximate amount of fermentable hexose sugars present. The fermentation method is employed almost entirely for determining small percentages of sugar, and has found its widest application in the determination of glucose in urine.

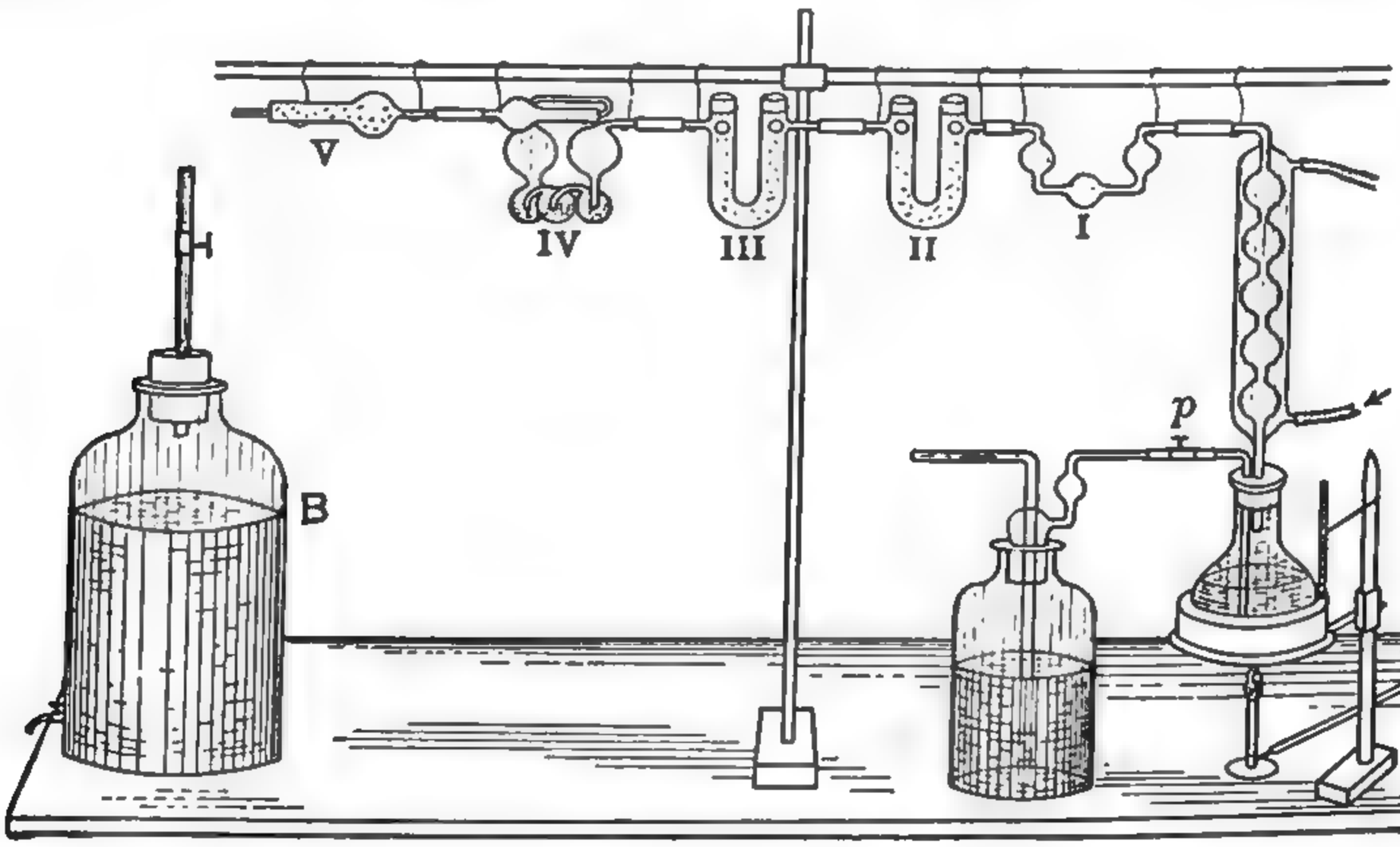


FIG. 298. Apparatus for determining sugars from weight of carbon dioxide given off by fermentation.

Direct Method by Weighing Carbon Dioxide. The most accurate method for determining the yield of carbon dioxide upon fermentation is shown in Fig. 298. A known amount of the solution is sterilized in a small flask, then cooled and inoculated with a pure culture of yeast. The flask is then connected by means of a condenser with a train of absorption tubes or bulbs. Bulb I (Fig. 298) contains a few milliliters of water, the U-tubes II and III contain calcium chloride for removing all moisture from the current of gas, the Liebig potash bulb

IV, which has been previously weighed, serves to absorb the carbon dioxide, and the safety tube V, containing calcium chloride and soda lime, prevents back absorption of water or carbon dioxide from the outside air. The fermentation is allowed to proceed either at room temperature, or, if desired, at 30° C., in which case the flask is immersed in a water bath carefully maintained at this temperature. At the end of 1 to 2 days, when no more gas passes through the bulb I, the tube V is connected with the aspirator bottle *B*, the pinchcock at *p*, which is previously closed, is opened, and a slow current of air, freed from carbon dioxide by passing through potassium hydroxide solution, is led through the apparatus. At the end of an hour the liquid in the flask is heated nearly to boiling, while a current of cold water circulates through the condenser; in this manner the last traces of dissolved carbon dioxide are expelled from the liquid. The aspiration is continued for another hour, when the potash bulb IV is disconnected and reweighed. The increase in weight gives the amount of carbonic acid.

The more usual process, in the fermentation method of estimating sugars, is to estimate the carbon dioxide by measuring the volume of gas; 1 ml. of evolved carbon dioxide (at 0° C. and 760-mm. atmospheric pressure) corresponds to 1.96 mg. carbon dioxide or about 4.2 mg. of glucose. For determining sugars by this method special forms of apparatus known as fermentation saccharometers have been devised, of which the three forms devised by Einhorn, by Lohnstein, and by van Iterson and Kluyver are selected as examples.

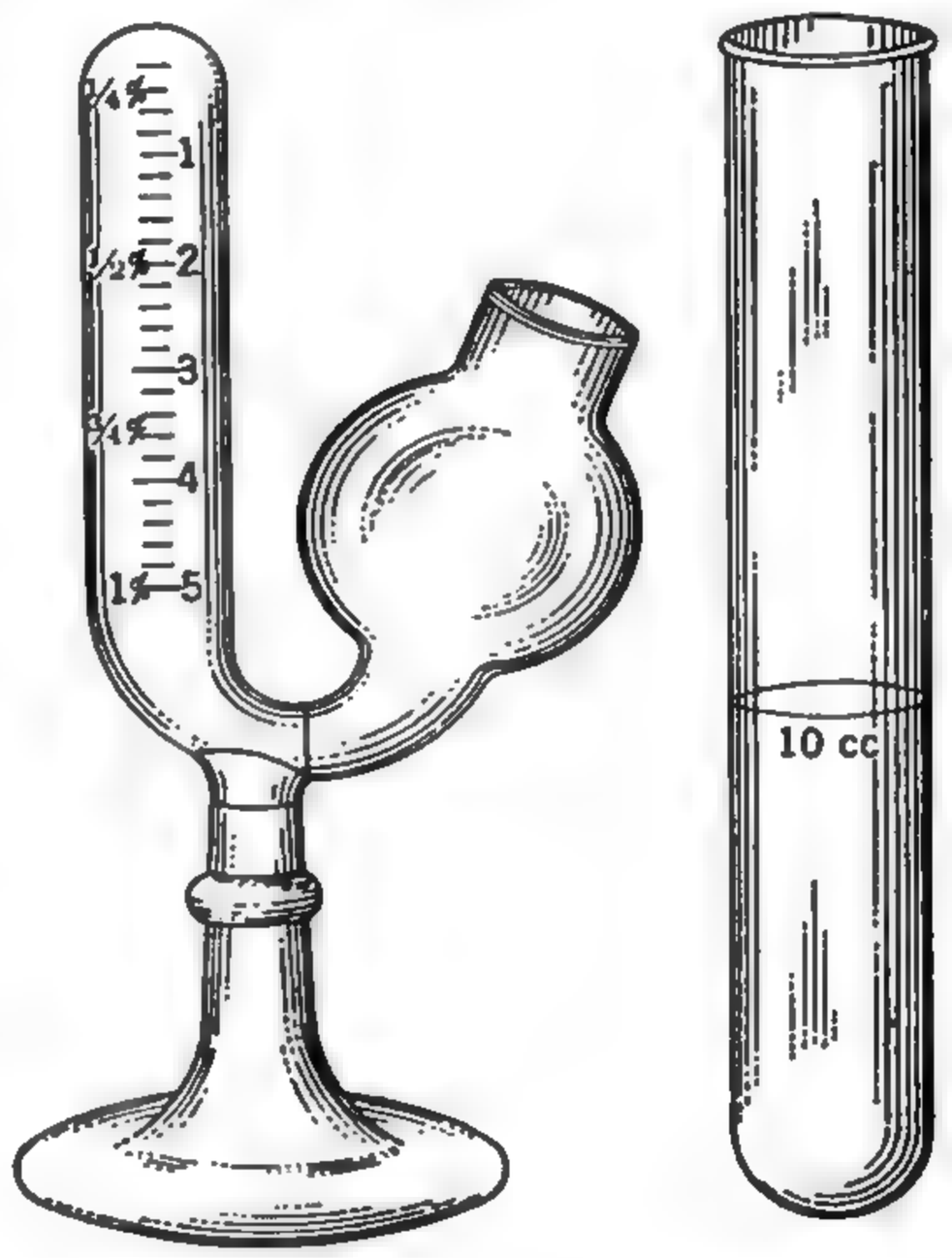


FIG. 299. Einhorn's fermentation saccharometer.

Einhorn's Fermentation Saccharometer.[139] This apparatus, which is designed for the estimation of small amounts of glucose in diabetic urine, is shown in Fig. 299. One gram of commercial pressed yeast is shaken thoroughly in the graduated test tube with 10 ml. of the urine. The mixture is then poured into the bulb of the saccharometer, the apparatus being inclined so that the graduated tube is completely filled. The saccharometer is then set

[139] Circular of information.

aside for 20 to 24 hours at ordinary temperature. If the urine contains sugar, fermentation will usually begin in about 30 minutes. When the fermentation is finished the volume of gas is measured in the graduated tube, the divisions of which indicate milliliters of gas and also the approximate fractions of per cent glucose. If the urine contains more than 1 per cent glucose it must first be diluted with water, the reading of the saccharometer being then multiplied by the degree of dilution. For diabetic urines of straw color and a specific gravity of 1.018 to 1.022 it is recommended to dilute twice; of 1.022 to 1.028 sp. gr. 5 times, and 1.028 to 1.038 sp. gr. 10 times.

Umbach[140] has found that the sugar scale of the Einhorn saccharometers on the market is often incorrectly calibrated, even though the graduation in milliliters is correct. This is due to the fact that the dimensions of the tubes vary, and that consequently the volume of liquid between the top of the graduated leg and the center of the U-bend is not the same. It is therefore advisable to check each apparatus with pure glucose solutions of known concentration.

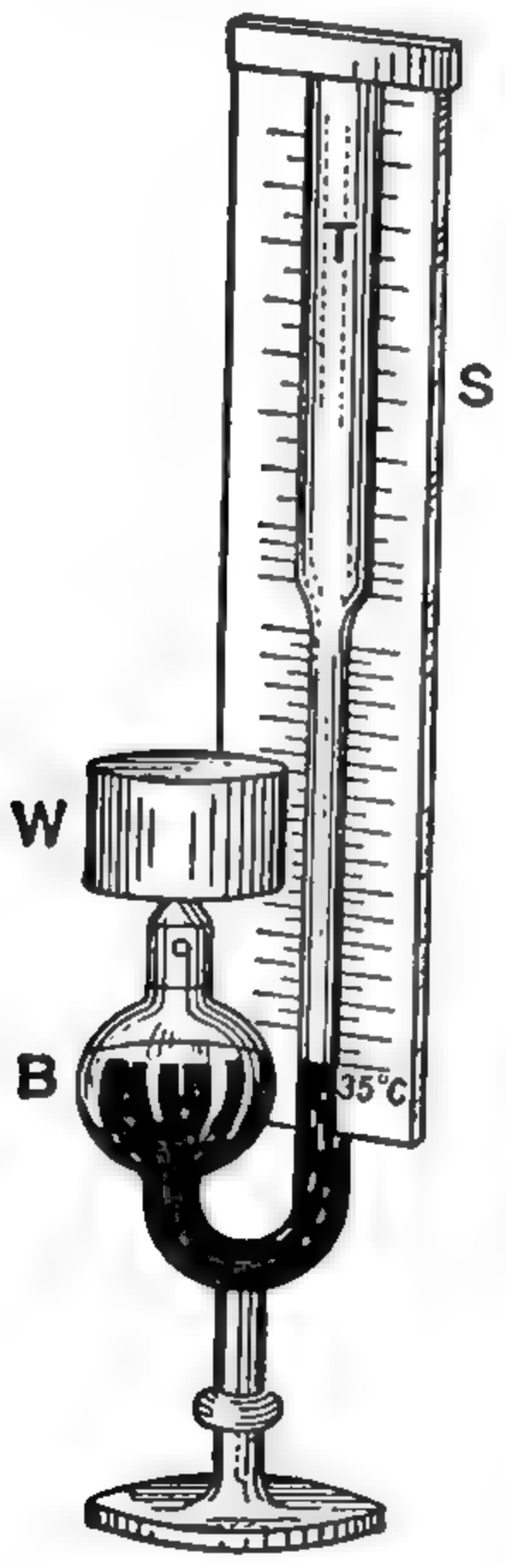


Fig. 300. Lohnstein's fermentation saccharometer.

It is always desirable in making the test to make a duplicate determination upon a normal urine. The latter should show at most only a small bubble of gas at the top of the tube; should a larger amount of carbon dioxide be obtained with normal sugar-free urine, the yeast is probably impure and the determination should be repeated. If the suspected urine shows no more gas than the control experiment the absence of glucose is indicated.

Lohnstein's[141] Fermentation Saccharometer. In Lohnstein's saccharometer (Fig. 300) the liquid is fermented over mercury in a closed bulb; the carbon dioxide which is evolved forces the mercury into an upright tube, the amount of displacement indicating the percentage of glucose present.

In making a determination the detachable scale *S* is hung in position over the open end of the tube *T*, and a quantity of mercury poured into the bulb *B* until its level in the tube is just opposite the zero mark of the scale. The standard weight of mercury necessary for the adjustment accompanies each instrument.

[140] *Chem.-Ztg.*, **52**, 273 (1928).

[141] *Münch. med. Wochschr.*, **46**, 1671 (1899).

A small piece of fresh baker's yeast is rubbed with 2 to 3 times its volume of ordinary water to a thin paste; 0.5 ml. of the urine, or other liquid to be tested, is then measured with a special pipette into the bulb; the pipette is rinsed into the bulb with a little ordinary water and a quantity of yeast suspension containing at least 50 mg., better 100 mg., of yeast is added. The glass stopper, which should be evenly greased, is then inserted and turned so that the small opening on its inner surface comes directly opposite a similar opening in the stem of the bulb. Any pressure of air due to inserting the stopper is thus released. The stopper is again slightly turned, so as to seal the contents of the bulb hermetically, and then securely fastened by the weight *W*. The apparatus is then set aside until fermentation is finished, which is indicated by the stationary position of the mercury column. The length of time necessary for completing the test will depend upon the temperature but does not ordinarily exceed 1 day at 20° C.; if an incubator is available the time may be shortened considerably by fermenting at 35° C. When fermentation is finished the scale division opposite the top of the mercury column indicates the percentage of sugar; for percentages of sugar below 2.0 the scale may be read to 0.01 per cent, and for percentages between 2.0 and 10.0 to 0.05 per cent. The scale is calibrated upon one side for 20° C. and upon the other for 35° C.; if the readings are made at intermediary temperatures the percentage of sugar is calculated by interpolating. Thus:

The reading of the mercury column at 25° C. was 4.0 on the 20° C. scale and 3.6 on the 35° C. scale. The corrected percentage of sugar is then $3.6 + \frac{4.0 - 3.6}{35 - 20}(35 - 25) = 3.87$ per cent.

A correction must be applied if the barometric pressure at which the readings are made differs appreciably from 760 mm. The following formula is used:

$$\text{Corrected per cent sugar} = \text{Per cent found} \times \frac{B + 90}{850}$$

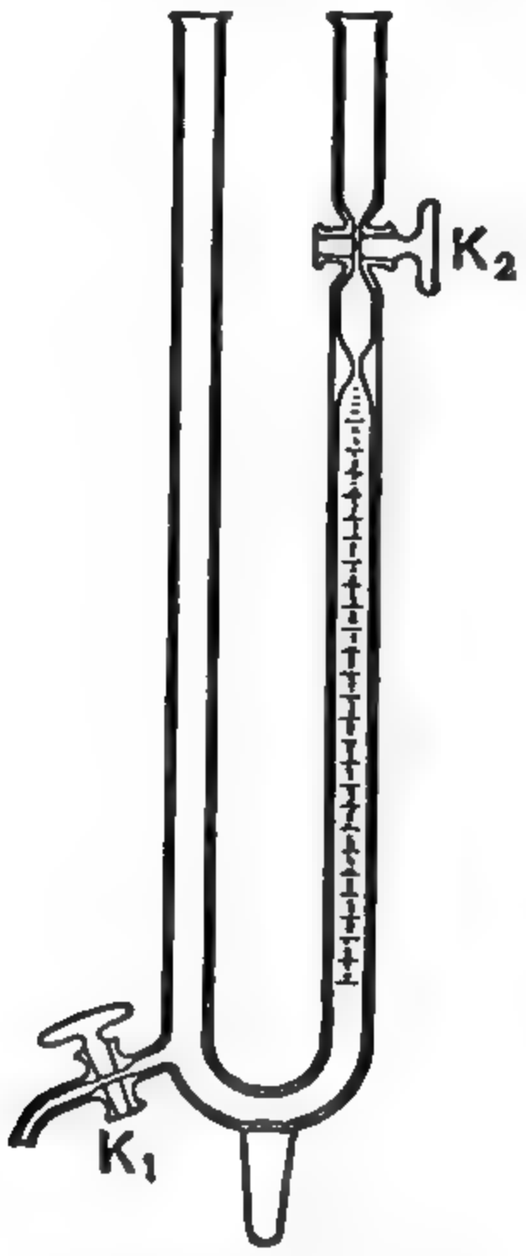
where B is the barometric pressure in millimeters.

It is also advisable to run a blank with the yeast suspension alone and to apply a correction for the apparent sugar content of the yeast.

Van der Haar[142] has found that with glucose, fructose, or mannose the error is within 0.25 to 1.5 mg. of the amount taken. Galactose is fermented much more slowly than the other three hexoses, and this makes it possible to determine any of the latter in the presence of

[142] "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide," p. 108, 1920.

galactose. The fermentation is carried out at 35° C.; at this temperature glucose, fructose, and mannose are completely fermented in 3 to 4 hours, and the mercury ceases to rise, while galactose yields no carbon dioxide under these conditions.



(Reproduced with permission from van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide," p. 110.)

FIG. 301. Fermentation apparatus of van Iterson-Kluyver.

Van Iterson-Kluyver Fermentation Method.[143] The apparatus of van Iterson-Kluyver makes it possible to use pure culture yeasts under sterile conditions, and for this reason it is better adapted for the analysis of sugar mixtures than the Lohnstein saccharometer. The apparatus is shown in Fig. 301 and needs no further description. It is sterilized before use by shutting stopcock K_1, closing the open ends of the U-tube with cotton wads, and placing the apparatus in a drying oven heated to 160° C. After cooling, stopcock K_1 being closed, sterilized mercury is poured carefully into the left leg of the U-tube until it reaches a little above K_2. Then 1 to 2 ml. of the sample containing about 2 per cent fermentable sugar is placed on top of the mercury, above K_2, and the cotton wad is replaced at once. The apparatus is inclined until the liquid above the mercury almost reaches the cotton wad. K_2 is now closed, and the sample is inoculated by means of a sterilized thick platinum wire with the pure yeast culture. After the cotton wad has been put on again, the apparatus is placed in a vertical position and K_2 is opened. Then mercury is run out through K_1 until the inoculated sample is partly above, partly below, K_2. The apparatus is again inclined until the meniscus of the mercury coincides exactly with the 1-ml. mark. K_2 is now closed again tightly. K_1 is opened and mercury is run out until it stands very low in order that the gas evolved may not be under pressure. The apparatus is then placed in an incubator at 30 to 35° C. During the fermentation the apparatus is carefully shaken from time to time, in order to prevent supersaturation of the liquid with carbon dioxide. The level of the mercury is kept low by removing more mercury through K_1. When the fermentation is completed the apparatus is taken out and kept at room temperature for half an hour, and the volume of the carbon dioxide is read after enough mercury has been added so that it stands at the same level in both legs of the U-tube. The temperature and barometric pressure are observed at the same time, and the volume

[143] Kluyver, Dissertation, Delft, 1914. See also van der Haar, *op. cit.*, p. 109.

is corrected to the standard of 0° C. and 760 mm. pressure by means of the figures given in Table CXXIV.

TABLE CXXIV

VOLUME PER CENT TO BE DEDUCTED FROM THE GAS VOLUME FOUND AFTER FERMENTATION IN THE VAN ITERSON-KLUYVER APPARATUS

Temperature, °C.	Barometric Pressure in Millimeters of Mercury					
	730	740	750	760	770	780
11	7.7	6.4	5.1	3.9	2.6	1.3
12	8.0	6.7	5.4	4.2	2.9	1.7
13	8.3	7.1	5.8	4.5	3.3	2.0
14	8.6	7.4	6.1	4.9	3.6	2.4
15	8.9	7.7	6.4	5.2	3.9	2.7
16	9.3	8.0	6.8	5.5	4.3	3.0
17	9.6	8.3	7.1	5.9	4.6	3.4
18	9.9	8.6	7.4	6.2	4.9	3.7
19	10.2	9.0	7.7	6.5	5.3	4.0
20	10.5	9.3	8.0	6.8	5.6	4.4
21	10.8	9.6	8.3	7.1	5.9	4.7
22	11.0	9.8	8.6	7.4	6.2	4.9

A further correction must be applied for the carbon dioxide which remains dissolved in the 1 ml. of liquid. This amounts, according to Kluyver, to 1.2 ml. at 0° C. and 760 mm. pressure. This is added to the carbon dioxide actually found.

Kluyver used pure cultures of the following yeasts in his investigations: *Saccharomyces cerevisiae* from both baker's and bottom yeast; *Torula dattila* from dates; lactose yeast, from buttermilk; and *Schizosaccharomyces Pombe*. He found that 1 ml. of carbon dioxide, at 0° C. and 760 mm. pressure, corresponds to the milligrams of sugar given in Table CXXV.

TABLE CXXV

MILLIGRAMS OF SUGAR CORRESPONDING TO 1 ml. CARBON DIOXIDE

	d-Glucose	*d*-Fructose	*d*-Mannose	*d*-Galactose
Saccharom. cerev., Baker's yeast	4.0	4.1	4.0	4.3 in 60 hours 4.1 in 5 days
Saccharom. cerev., Bottom yeast	4.1	4.1	4.0	
Torula dattila	4.0	4.1	4.1	...
Lactose yeast	4.2	4.2	4.3	4.4
Schizosacch. Pombe	4.2	4.2	4.2	...

Torula dattila and *Schizosaccharomyces Pombe* do not ferment galactose at all; the other organisms require 60 hours or more to complete the fermentation of this sugar. Glucose, fructose, and mannose are completely fermented in about 3 hours by *Saccharomyces cerevisiae* or by the lactose yeast.

If mixtures of any or all of the last three sugars with galactose are to be analyzed, the sum of all of them is determined by fermenting with a pure culture of baker's yeast for at least 60 hours, and the sum of glucose, fructose, and mannose by fermentation with *Torula dattila* or *Schizosaccharomyces Pombe.* It is also possible to combine determinations by the van Iterson-Kluyver apparatus and the Lohnstein saccharometer. In this case only one pure culture is required. For instance, the sum of glucose, fructose, and mannose may be found by fermentation for 3 hours at 35° C. in the Lohnstein apparatus, and the sum of the four sugars by fermentation with pure lactose yeast in the van Iterson-Kluyver apparatus for 60 hours or more. In either procedure the galactose is found by difference. The following example of the necessary calculations is cited from van der Haar:

Example. An aqueous solution containing 100 mg. each of glucose and galactose in 10 ml. at 15° C. was prepared, and 0.5 ml. of the solution was fermented with 100 mg. ordinary pressed yeast for 3 hours at 35° C. in the Lohnstein apparatus. The readings at 15° C. and 735 mm. pressure were 1.26 on the 20° C. scale, and 1.04 on the 35° C. scale. The percentage of sugar was therefore

$$1.04 + \frac{1.26 - 1.04}{35 - 20} \times (35 - 15) = 1.333 \text{ per cent}$$

Corrected to 760 mm. pressure, the actual percentage of sugar is

$$1.33 \times \frac{735 + 90}{850} = 1.293$$

The 0.5-ml. solution contained therefore 6.465 mg. glucose, from which the blank of 1 mg. glucose must be deducted, giving 5.465 mg. glucose, against 5 mg. actually used.

In the van Iterson-Kluyver apparatus 1 ml. of solution is used. This 1 ml. contains 2×5.465 mg. = 10.93 mg. glucose. Since 4.2 mg. glucose corresponds to 1 ml. carbon dioxide, the 10.93 mg. correspond to $10.93/4.2 = 2.6$ ml., which must be deducted from the total carbon dioxide found after fermentation in the van Iterson-Kluyver apparatus.

Fermentation in the van Iterson-Kluyver apparatus with pure lactose yeast for 72 hours gave 3.95 ml. carbon dioxide at 735 mm. and 17.5° C. According to Table CXXIV, 9.1 per cent of the volume must be deducted, or 0.36 ml., leaving 3.59 ml. This volume must again be increased by 1.2 ml. for

the carbon dioxide remaining in solution at the end of the fermentation. The corrected carbon dioxide from the two sugars is therefore 3.59 + 1.20 = 4.79 ml. If the 2.6 ml., corresponding to the glucose alone, as found in the Lohnstein apparatus, is deducted, there remains 2.19 ml. formed from the galactose. This, multiplied by 4.4 (Table CXXVI), gives 9.64 mg. galactose in 1 ml. solution, against 10 mg. actually used. It is noted that the result for glucose is slightly too high, and that for galactose slightly too low.

It is also possible to remove the glucose, fructose, and mannose by 3-hour fermentation, and to determine the galactose remaining by a copper or other reduction method.

Van Slyke and Hawkins[144] have employed the Van Slyke-Neill manometric apparatus[145] for measuring the carbon dioxide formed from the fermentable sugars in blood and urine.

Instead of finding the weight or volume of carbon dioxide the percentage of fermentable sugar may also be calculated from the amount of alcohol which is formed by the action of yeast, or from the difference in specific gravity of the solution before and after fermentation. A valuable check upon the accuracy of the results obtained by the fermentation methods is to determine the loss in reducing sugars by means of Fehling's solution.

Similarly, the presence of reducing non-sugars may be ascertained by determining the reducing power of the solution before and after fermentation with yeast. This method is used particularly with biological materials, as blood, urine, glycogen, etc., and also to determine "glutose" in molasses (p. 957).

Somogyi's Fermentation Method for Determining True Sugar Values in Blood, etc.[146] It has been pointed out on pp. 888–893 that the clarifying agents used prior to the determination of reducing sugars remove reducing non-sugars to a varying extent. Somogyi recommends, therefore, that the reducing power be determined before and after fermentation of the clarified filtrates. The fermentation is carried out with a large proportion of yeast in a short time, because otherwise the yeast gives rise to the formation of reducing non-sugars.

In blood-sugar analysis, 6 to 7 ml. of a 20 per cent suspension of carefully washed yeast is placed in a Pyrex tube. The suspension is centrifuged and the supernatant liquid discarded. The tube is inverted and allowed to drain for a few seconds. The moisture remaining on the tube wall is removed with a wad of filter paper. Somogyi found that the yeast prepared in this manner contains so little moisture

[144] *J. Biol. Chem.*, 83, 51 (1929).
[145] *J. Biol. Chem.*, 61, 523 (1924).
[146] *J. Biol. Chem.*, 78, 117 (1928).

that it does not affect the results. From 12 to 14 ml. of the deproteinized blood filtrate is transferred to the tube containing the yeast and thoroughly mixed with it by stirring with a glass rod. The mixture is allowed to stand at 25 to 30°C. for about 10 minutes, the tube being inverted from time to time to keep the yeast in suspension. The contents of the tube are then transferred to another Pyrex test tube without moistening its walls. This tube is centrifuged, the clear liquid decanted immediately, and the reducing power is determined, in this solution as well as in the unfermented solution. The difference between the two values gives the true blood sugar. In the analysis of urine, glycogen hydrolysates, etc., the concentrations are modified to suit the particular conditions.

Determination of Lactose in the Presence of Other Carbohydrates. The fact that baker's yeast does not ferment lactose to any large extent is made use of in a method devised by Magraw, Copeland, and Sievert[147] for determining lactose in complex mixtures as mixed feeds. All the other carbohydrates present are converted into fermentable sugars by means of enzymes, and after fermentation the lactose remaining is estimated by copper reduction. The method is carried out as follows:

Place 16.25 g. of the well-mixed feed in a 300-ml. volumetric flask with about 200 ml. of distilled water and digest in a hot-water bath, with occasional shaking, for a period of 30 minutes. Cool, fill to volume with distilled water, and centrifuge.

Place 150 ml. of the supernatant solution in a 200-ml. volumetric flask. Add 0.25 g. of animal diastase (Armour & Co., Pharmaceutical Department) and place in a constant-temperature bath at 52° to 55° C. for 25 to 30 minutes. Place the flask in a boiling-water bath for 15 minutes. Cool the flask, add another 0.25 g. of the animal diastase and repeat the incubation and subsequent heating as before. After the flask has been cooled to room temperature, add 75 mg. of invertase-melibiase scales (Nulomoline Co., 120 Wall St., New York, N. Y.) and 1.5 to 2 g. of baker's yeast, and plug the flask with sterile cotton. After fermentation at 26.5 to 30° C. (not above 33°), for a period of 17 to 18 hours, make up to volume and centrifuge. Reduce 190 ml. of the supernatant liquid to 50 ml. by boiling and wash into a 100-ml. volumetric flask with the aid of hot distilled water. Add 10 ml. of saturated neutral lead acetate solution, make up to volume, and centrifuge. To 50 ml. of clear liquid in a 100-ml. volumetric flask add 2.5 ml. of 5 per cent solution of mercuric chloride and allow to stand 15 minutes, with repeated shaking. Then add 5 ml. of a 20 per cent solution of phosphotungstic acid. Make up to volume with distilled water and remove the precipitate by centrifuging. If the solution is not clear after centrifuging, it should be filtered through a dry

147 *J. Assoc. Official Agr. Chem.*, **19**, 605 (1936).

filter paper. Saturate the resulting liquid with hydrogen sulfide and filter. Pipette 50 ml. of the clear, colorless solution into a 400-ml. beaker, mark the level, and boil to remove the excess hydrogen sulfide. Add water to restore the volume to 50 ml., and determine the lactose by the Munson and Walker method.

The 50 ml. solution in which the lactose is determined contains 2 g. of the original sample, i.e., $16.25 \times \frac{150}{(300 - 8)} \times \frac{190}{200} \times \frac{50}{100} \times \frac{50}{(100 - 1)}$ g. The figures 8 and 1 allow for the volume of the sample and of the clarification precipitate, respectively. The percentage of lactose in the feed is then calculated by the formula:

$$\text{Per cent lactose} = \frac{100(X - 0.006)}{0.96 \times 2.00}$$

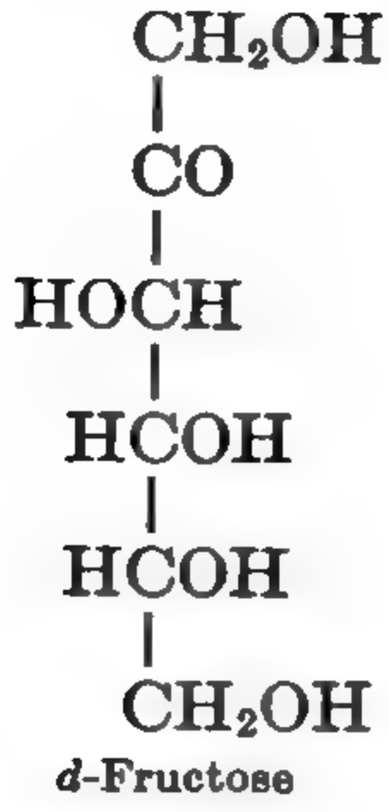
where X is the grams lactose found, 0.006 is a correction for the blank, 0.96 the factor allowing for 4 per cent loss of lactose by fermentation, and 2.00 the grams of sample in the aliquot used for analysis. The correction of 0.006 is an average of the results of many analyses made with lactose-free samples. Lactose added to such samples was recovered within ± 0.2 per cent.

Similar methods have been described for the determination of lactose in bread by Snethlage[148] and by Hoffman, Schweitzer, and Dalby,[149] and for the determination of lactose in milk chocolate by van Voorst.[150]

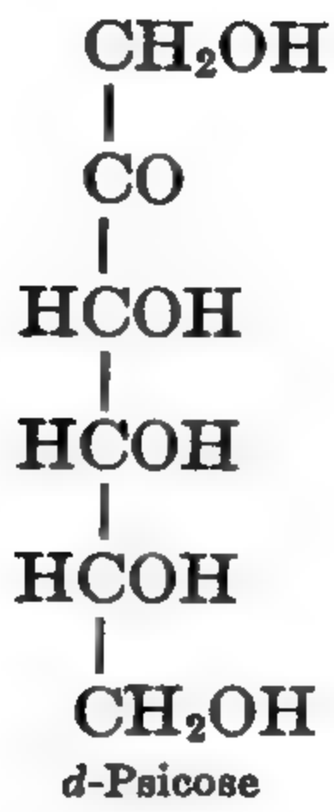
Determination of Unfermented Reducing Substances in Molasses. The reducing substance remaining in molasses after fermentation with yeast was considered by Lobry de Bruyn and Alberda van Ekenstein[151] to be identical with glutose because it is formed under conditions similar to those by which they obtained this sugar from glucose or fructose. Glutose is supposed to be a 3-ketohexose, but its structure and configuration have not been established. Many unsuccessful attempts had been made to identify the unfermented reducing substance in molasses until Sattler, at the New York Sugar Trade Laboratory, obtained from the unfermentable residue of a molasses distillery the osazone of a 2-ketohexose which has been identified as *d*-psicose (*d*-pseudofructose), previously synthesized by Steiger and Reichstein.[152] This sugar is the

[148] *Chem. Weekblad*, 23, 578 (1926).
[149] *Ind. Eng. Chem., Anal. Ed.*, 8, 298 (1936).
[150] *Chem. Weekblad*, 34, 803 (1937).
[151] *Rec. trav. chim.*, 16, 274 (1897); 18, 72 (1899).
[152] *Helv. Chim. Acta*, 19, 184 (1936).

epimer of *d*-fructose:



d-Fructose



d-Psicose

Other sugars besides *d*-psicose appear to be present in the unfermented molasses residue.

Methods for the determination of unfermented reducing substances in molasses have been devised by Pellet,[153] by Waterman and van der Ent,[154] and by others. The Java Sugar Experiment Station[155] has adopted the following procedure, which is a modification of earlier methods. Twelve grams of final molasses is transferred with 75 ml. of water to a large Erlenmeyer flask (about 600-ml. capacity), and 25 g. of fresh baker's yeast is added. After thorough mixing the fermentation is allowed to proceed at 30° C. for at least 4 hours. The mixture is then washed quantitatively into a 250-ml. flask, clarified with 25 ml. of 10 per cent neutral lead acetate solution, and the flask is filled to the mark with water. One-half gram of kieselguhr is added, and the well-shaken mixture is filtered, the first portions of the filtrate being discarded. Fifty milliliters of the filtrate is pipetted into a 100-ml. flask, and the excess lead is removed by the addition of 5 ml. of a solution containing 7 g. of crystallized disodium phosphate and 3 g. of potassium oxalate in 100 ml. The volume is completed to the mark and the solution filtered; 25 ml. of the filtrate is mixed with 20 ml. of combined Soxhlet solution in a 300-ml. Erlenmeyer flask, and the walls of the flask are washed down with 5 ml. of water, making 50 ml. in all. A few pieces of pumice stone are added, and the flask is placed on a wire gauze covered with an asbestos plate which has a hole in the center, slightly smaller than the bottom of the flask. The liquid is heated to boiling in 3 minutes, and then gently boiled for exactly 2 minutes longer. It is then quickly cooled, 15 ml. of 20 per cent potassium iodide solution is added, and then 10 ml. of

[153] *Bull. assoc. chim. sucr. dist.*, **35**, 116, 178 (1917/18).
[154] *Arch. Suikerind.*, **34**, 942 (1926).
[155] "Methoden van Onderzoek," 6th ed., p. 365, 1931.

dilute sulfuric acid (1 volume concentrated acid plus 5 volumes water). The liberated iodine is titrated with *N*/10 thiosulfate solution, 2 ml. of a 1 per cent starch solution being added toward the end of the titration. A blank experiment is run with 75 ml. of water. The titer of the sample is deducted from that of the blank, and the milligrams of reducing sugar, expressed as invert sugar, are found from Table CXXVI. The resulting figure, divided by 6, gives the percentage of unfermented reducing substance in the molasses, expressed as invert sugar. Since glutose is supposed to have only one-half the reducing power of invert sugar, the result, multiplied by 2, gives per cent glutose.

TABLE CXXVI

Milligrams of Invert Sugar Corresponding to Milliliters of *N*/10 Thiosulfate

N/10 Thiosulfate	Invert Sugar	*N*/10 Thiosulfate	Invert Sugar
ml.	mg.	ml.	mg.
1	3.2	14	47.3
2	6.4	15	50.8
3	9.7	16	54.3
4	13.0	17	58.0
5	16.4	18	61.8
6	19.8	19	65.5
7	23.2	20	69.4
8	26.5	21	73.3
9	29.9	22	77.2
10	33.4	23	81.2
11	36.8	24	85.2
12	40.3	25	89.2
13	43.8	..	....

Any other convenient method may, of course, be used for the determination of the reducing power.

If any appreciable quantities of unfermentable reducing substances are present in molasses, its invert-sugar content, as found by the usual copper-reduction methods, is obviously too high. It should be corrected by deducting from the total percentage of invert sugar the unfermentable sugars, expressed as invert sugar.

Davis[156] has described a method for the determination of unfermented reducing substances, which is patterned after actual distillery practice and requires at least a week for completion. It is not suitable for routine commercial analyses.

[156] "Methods of Analysis of Molasses Used in the Fermentation Industries," published by the Research Laboratories of the Distillers Company, Ltd., Epsom, England, 1938; *Intern. Sugar J.*, 40, 186, 235 (1938).

COLORIMETRIC METHODS FOR DETERMINING SUGARS

Colorimeters and the technique of using them have been described in Chapter XII, and several methods in which colorimetric comparisons are employed have been referred to on previous pages. It remains to give some further examples of procedures, based on this principle, for the direct estimation of sugars. Such a method was first devised by Dubrunfaut, who determined small percentages of glucose by comparing the color which was produced by heating the solution with alkalies with the colors of solutions containing known amounts of pure glucose which had been similarly treated.

In addition to the alkalies many of the special reagents used in making color and spectral reactions, such as α-naphthol and resorcinol, have been employed for the colorimetric estimation of sugars. The principal requirement in the use of such reagents for quantitative purposes is that the color produced must be perfectly soluble and of a fair degree of stability. The insoluble or evanescent colors which are produced in many of the reactions for sugars are valueless for colorimetry.

Example. Fifty grams of a glucose solution of unknown strength was made up to 500 ml. with water, and 5 ml. of dilute sodium hydroxide solution added (solution I).

One gram of pure glucose was dissolved in water and the solution made up to 500 ml., 5 ml. of the same sodium hydroxide solution being also added (solution II).

Both solutions were heated in a hot-water bath for the same length of time and after cooling compared in a Duboscq colorimeter.

The following results were obtained:

Reading Solution I	Reading Solution II	Computations
50 mm.	80.1 mm.	$\frac{80.1 \times 1}{50} = 1.602$
40 mm.	63.7 mm.	$\frac{63.7 \times 1}{40} = 1.593$
35 mm.	55.9 mm.	$\frac{55.9 \times 1}{35} = 1.597$
		Average 1.597

The unknown solution contained therefore 1.60 g. glucose in 500 ml. of solution, or 3.2 per cent in the original sample.

The color reactions of sugars, described in Chapter XIII, are usually not strictly specific, and are furthermore affected by the presence of organic or mineral impurities. The application of colorimetric methods in the analysis of complex mixtures is for this reason largely curtailed.

But in certain cases, especially with biological materials, they have been found useful because often only small quantities of sample are available and the usual methods fail to give results. A few examples of such colorimetric methods will be described.

Diphenylamine Method for Fructose. This procedure is based on the blue coloring matter which diphenylamine gives with the hydroxymethylfurfural resulting from fructose upon heating in the presence of strong acids. The method of van Creveld,[157] as modified by Oppel,[158] is selected for description. Standards containing from 0.1 to 0.5 mg. of fructose in 1 ml. are prepared, and the suitably clarified sample is diluted so that its fructose concentration falls within that range. One milliliter each of the sample and standards is placed in test tubes, 0.1 ml. of a 20 per cent solution of diphenylamine in alcohol is added to each, followed by 1 ml. of 25 per cent hydrochloric acid. The tubes are placed for 20 minutes in a vigorously boiling water bath, and then quickly cooled in running water. The coloring matter is extracted in each tube by shaking with 2.5 ml. isoamyl alcohol. After complete settling the isoamyl alcohol layer is pipetted off and diluted with 30 ml. of alcohol, and the sample is compared colorimetrically with the standards. The experimental error does not exceed 10 per cent.

Resorcinol Method for Fructose Determination in Blood and Urine. The deep red color produced when fructose is heated in strongly acid solution with resorcinol has also been utilized. According to Roe[159] the determination is carried out as follows. The reagent used is a solution of 0.5 g. resorcinol in 500 ml. of 95 per cent alcohol.

Fructose in Blood. One part of blood is mixed with 7 parts of water. After a few minutes the solution is clarified by the addition of 1 part of zinc sulfate solution (10 g. $ZnSO_4 \cdot 7H_2O$ dissolved to 100 ml.), and 1 part of 0.5 *N* sodium hydroxide. The precipitate is filtered off, and 2 ml. of the filtrate is placed in a test tube. The standards are prepared by dissolving fructose in saturated benzoic acid solution, at concentrations of 0.1, 0.05, and 0.025 mg. per ml. These standards keep for a long time. Two milliliters each of these standard solutions is pipetted into test tubes similar to that used for the blood sample. Then 2 ml. of the resorcinol reagent, and 6 ml. of 30 per cent hydrochloric acid (5 volumes concentrated acid and 1 volume of water) are added to each tube. The tubes are shaken and placed for 8 minutes in a water bath heated to 80° C. They are then immediately cooled, and the sample is compared colorimetrically with the standards.

[157] *Nederland. Tijdschr. Geneeskunde*, 70, II, 2779 (1926).
[158] *Biochem. Z.*, 229, 85 (1930).
[159] *J. Biol. Chem.*, 107, 15 (1934).

Fructose in Urine. Two milliliters of urine is diluted with 18 ml. of 1 per cent acetic acid. Then 0.2 g. of acid-washed activated carbon is added, the mixture shaken vigorously, and allowed to stand for 5 minutes with occasional agitation. It is then filtered, and 2 ml. of the filtrate is treated as described under blood. The standards are in this case prepared by dissolving fructose in 1 per cent acetic acid. If protein is present in the urine, it is first clarified with zinc sulfate and sodium hydroxide in the same manner as blood.

Glucose and galactose also produce small quantities of hydroxymethylfurfural, and cause a plus error. When the glucose concentration is 3 times that of the fructose the error amounts to 1 per cent, with 5 times 2.2 per cent, with 50 times 41.3 per cent. For galactose in 5-fold concentration the error is 1.4 per cent, 10-fold 4.0 per cent, 50-fold 27.6 per cent. Pentoses, when present in excess of 10 mg. in 1 ml. of urine, also interfere because the furfural formed produces a green coloration with resorcinol. Errors of this nature can sometimes be overcome by inserting suitable color filters in the colorimeter or by employing a monochromatic light source.

Method of Elson and Morgan for Determining Glucosamine and Chondrosamine.[160] These amino sugars condense with acetylacetone to pyrrole derivatives:

$$\underset{\text{Glucosamine}}{CH_2OH-(CHOH)_3-CHNH_2-CHO} + \underset{\text{Acetylacetone}}{CH_3-CO-CH_2-CO-CH_3} \rightarrow$$

$$CH_2OH-(CHOH)_3-\begin{array}{l}C(=CH-C(=CH-CO-CH_3)-)\\ \quad\quad\quad\

The unknown solution, which should contain between 0.5 and 3.0 mg. of glucosamine, is pipetted into a test tube marked at 10-ml. volume, and 1 ml. of the acetylacetone solution is added. The walls of the tube are washed down with 1 ml. of water. The standard solutions are measured out and treated in the same manner. The tubes are heated for 15 minutes in a boiling-water bath in such a way that the level of the water is just above that of the liquid in the tubes, but the upper portions of them project out of the bath, in order to avoid evaporation. The tubes are cooled, and alcohol is added to within about 2 ml. of the 10-ml. mark. One milliliter of the dimethylaminobenzaldehyde reagent is added to each tube, and the volume completed to 10 ml. with alcohol. The tubes are then allowed to stand for 30 minutes at room temperature, during which time evolution of carbon dioxide has stopped. The tube with the unknown is compared colorimetrically with the standards. For accurate comparisons the concentration of the unknown should not differ more than 25 per cent from the standard. The error of the estimation is less than 5 per cent. Chondrosamine is determined in the same way as glucosamine.

Sucrose, fructose, galactose, arabinose, glycine, alanine, and histidine do not interfere, but pyrrole or indole derivatives must be absent. The presence of the last two can be detected by the fact that they react with dimethylaminobenzaldehyde without previous addition of acetylacetone. Tryptophan does not react under the conditions used. *l*-Aminoglucose gives the reaction, but it has not been found so far in natural products.

Ehrlich's Colorimetric Method for Estimating Caramel. Ehrlich[161] has devised a colorimetric method for estimating caramel, in which the standard of comparison is saccharan. This dark-colored caramel substance is produced by heating sucrose in a flask immersed in oil to about 200° C. under vacuum. The residue, after extracting with boiling methyl alcohol, is dissolved in water, filtered, and evaporated. The saccharan, $C_{12}H_{18}O_9$, is obtained as a dark-brown residue (about 20 per cent of the weight of sucrose) which is easily pulverized to an amorphous powder. One part of saccharan in 10,000 of water colors the solution a deep brown, which is intensified by the addition of alkalies. Ehrlich found that saccharan is not precipitated by lead subacetate solution. He concluded that if the latter clarifying agent is used for precipitating other coloring substances from solutions of sugar, molasses, etc., the percentage of saccharan in the neutralized filtrate may be estimated by comparison in a colorimeter with a solution containing

[161] *Z. Ver. deut. Zucker-Ind.*, **59**, 746 (1909); *Proc. Seventh Intern. Congr. Applied Chem.*, Section V, p. 92.

a known weight of saccharan. Meade[162] has found, however, that the impurities which are present in molasses and other dark-colored sugar products, when precipitated by lead subacetate or even by neutral lead acetate, carry down with them all or nearly all of the caramel in the solution. Ehrlich's method is therefore valueless with these products and can be applied only to caramel solutions free from substances which are precipitated by lead.

DETERMINATION OF SUGARS AS HYDRAZONES AND OSAZONES

The varying solubility of the different hydrazones and osazones of sugars, or of similar derivatives, in the presence of impurities, has prevented the general employment for quantitative purposes of this means of separating sugars. In certain cases, however, where the hydrazone or osazone is characterized by great insolubility a fairly accurate determination of several of the sugars has been found possible.

Determination of Arabinose as Diphenylhydrazone. According to Neuberg,[163] arabinose is precipitated quantitatively by treating the sirupy solution of sugar with a slight excess of diphenylhydrazine. Sufficient alcohol is added to form a perfectly clear solution, and the mixture is heated to boiling for 30 minutes in a water bath in a flask connected with a reflux condenser. The solution is cooled and allowed to stand for several hours, and the white crystalline hydrazone is filtered into a weighed Gooch crucible. After washing with a few milliliters of cold alcohol, the crucible is dried in a water oven and weighed.

The weight of arabinose diphenylhydrazone, $C_5H_{10}O_4N{\cdot}N(C_6H_5)_2$, is calculated to arabinose, $C_5H_{10}O_5$, by multiplying by $\frac{150}{316} = 0.4747$. This method of analysis has been used by Neuberg for estimating arabinose in the urine and by Maurenbrecher and Tollens[164] for determining arabinose in cacao.

Wise and Peterson[165] have employed a slightly modified procedure in the analysis of hydrolyzed arabogalactan from western larch.

Fucose or large quantities of mannose also yield a difficultly soluble diphenylhydrazone, and these sugars should be absent if arabinose is to be determined by this method.

Determination of Mannose as Phenylhydrazone. The property of mannose in forming with phenylhydrazine a very insoluble hydrazone, discovered by Fischer and Hirschberger,[166] has been used for the

[162] *Ind. Eng. Chem.*, **15**, 275 (1923).
[163] *Ber.*, **35**, 2243 (1902).
[164] *Ber.*, **39**, 3578 (1906).
[165] *Ind. Eng. Chem.*, **22**, 362 (1930).
[166] *Ber.*, **21**, 1805 (1888).

quantitative estimation of mannose. The precipitation, according to Bourquelot and Hérissey,[167] is best accomplished by treating a 3 to 6 per cent solution of the sugar with an excess of phenylhydrazine acetate at a temperature not above 10° C. After standing 24 hours, the white crystalline hydrazone is filtered upon a weighed Gooch crucible, washed with a little cold water, dried in a water oven, and weighed. The solubility of the hydrazone is 0.04 g. in 100 ml. of solution, and the weight of precipitate should be corrected accordingly.

The weight of mannose phenylhydrazone, $C_6H_{12}O_5N_2HC_6H_5$, is calculated to mannose, $C_6H_{12}O_6$, by multiplying by $\frac{1}{2}\frac{8}{7}\frac{0}{0} = \frac{2}{3}$, or 0.6666. The method is well adapted for determining mannose in the presence of other sugars and has been employed by Pellet[168] for estimating small amounts of mannose in sugar-cane molasses.

Determination of Mannan. The same reaction may also be utilized for the determination of mannan, after previous hydrolysis.[169] Ten grams of the material to be analyzed is boiled under reflux with 150 ml. of 5 per cent hydrochloric acid (sp. gr. 1.025) for several hours; 3 to 4 hours is usually sufficient. The mixture is filtered; the residue is washed back into the flask with about 100 ml. of water, digested for a few minutes on the hot plate, and filtered again. This digestion process is repeated until about 500 ml. of total filtrate has been obtained. The solution is neutralized with sodium hydroxide, slightly acidified with acetic acid, and evaporated to a volume of about 150 ml. It is filtered into a glass-stoppered Erlenmeyer flask, and 10 ml. of phenylhydrazine and 20 ml. of water acidified with glacial acetic acid are added. After several hours' standing at a low temperature with frequent shaking the phenylhydrazone is filtered off on a Gooch crucible fitted with a disk of mercerized cotton cloth, washed with cold water and then with acetone, and dried at 100°. The weight of the residue, corrected as described above, is multiplied by 0.6 (i.e., 0.6666×0.9), to obtain the weight of the mannan originally present.

Determination of Fructose as Methylphenylosazone. According to Neuberg,[170] fructose may be determined with a fair approximation by precipitating as its methylphenylosazone, $C_6H_{10}O_4(N_2CH_3C_6H_5)_2$. About 10 ml. of the concentrated sugar solution is treated with a slight excess of methylphenylhydrazine, and sufficient alcohol added to give a clear solution. If other sugars than fructose are present the solution

[167] *Compt. rend.*, 129, 339 (1899).
[168] *Bull. assoc. chim. sucr. dist.*, 16, 1181 (1898/99); 18, 758 (1900/01).
[169] Schorger, *Ind. Eng. Chem.*, 9, 748 (1917); Dore, *Ind. Eng. Chem.*, 12, 476 (1920). See also Nowotnowna, *Biochem. J.*, 30, 2177 (1936).
[170] *Ber.*, 35, 960 (1902).

is slightly warmed and allowed to stand 24 hours for the separation of any insoluble hydrazones of mannose, galactose, etc. After any precipitate is removed by suction, the filtrate is treated with 4 ml. of 50 per cent acetic acid, heated 5 to 10 minutes upon the water bath, and then set aside in the cold for 24 hours. The reddish yellow crystals of the osazone are filtered in a weighed Gooch crucible and calculated to fructose, $C_6H_{12}O_6$, by multiplying by $\frac{180}{386} = 0.4663$. The method is only approximate as 10 per cent or more of the osazone remains in solution. By using a very cold freezing mixture the separation has been made almost quantitatively.

Volumetric Determination of Sugars by Means of the Osazones. Knecht and Hibbert[171] discovered that glucosazone is reduced by titanium trichloride stoichiometrically to isoglucosamine, which Fischer had previously obtained by reduction with zinc dust and acetic acid. It has been identified as 1-amino fructose, $CH_2OH—(CHOH)_3—CO—CH_2NH_2$. The reduction with titanium trichloride takes place according to the equation

$$C_{18}H_{22}O_4N_4 + 6TiCl_3 + 9HCl + H_2O \longrightarrow C_6H_{13}NO_5 + 2C_6H_5NH_2, HCl + NH_4Cl + 6TiCl_4$$

One molecule of glucose, in the form of its phenylosazone, requires 6 molecules of titanium trichloride, equivalent to 6 atoms of hydrogen, for its reduction to isoglucosamine.

The usual methods for the preparation of phenylosazones do not give quantitative yields, but if 12 molecules of phenylhydrazine are used to 1 molecule of sugar, the conversion into the osazone is complete even though part of it remains in solution. Phenylhydrazine itself is not reduced by titanium trichloride. Knecht and Hibbert have based on the foregoing facts the following method for determining sugars which form osazones:[172]

Preparation and Standardization of the Titanium Trichloride Solution. Fifty milliliters of commercial 20 per cent titanium trichloride is boiled in a small flask with 100 ml. concentrated hydrochloric acid for 1 minute, and made up to a volume which will almost completely fill a bottle holding about 2.25 liters, serving as a reservoir for an automatic burette. The burette is filled through a tube with pinchcock, passing from a side arm near the lower end of the burette to an outlet near the bottom of the bottle. The air space above the liquid in the burette is connected with that in the bottle by means of a glass tube passing

[171] *J. Chem. Soc.*, 125, 2009 (1924).
[172] "New Reduction Methods in Volumetric Analysis," pp. 62, 115, 1925.

through rubber stoppers. Through another hole in the rubber stopper on the top of the bottle, the space above the liquid in the bottle is connected with a small hydrogen generator. Whenever liquid is withdrawn from the system, the generator operates automatically and keeps the titanium trichloride always under an atmosphere of hydrogen. These arrangements are necessary because the titanium chloride is rapidly oxidized by the oxygen of the air.

The titanium trichloride solution is standardized with ferric salt; 3.5 g. of ferrous ammonium sulfate (Mohr's salt) is dissolved in 100 ml. of 5 *N* sulfuric acid, and the volume is made up to 250 ml. Twenty-five milliliters of this solution, containing 0.050 g. metallic iron, is completely oxidized to the ferric state with *N*/50 potassium permanganate solution, added slowly until the faintest pink is observed. A large excess of potassium thiocyanate is added, and the titanium trichloride solution is run in until the red color of the ferric thiocyanate complex is discharged. If it took 26.3 ml. of titanium trichloride solution to reduce the ferric salt solution, 1 ml. of the titanium solution is equivalent to $0.050 \div 26.3 = 0.001901$ g. iron.

Determination. To 10 ml. of a solution containing 0.01 to 0.02 g. sugar, e.g., glucose, add 1 ml. of a saturated solution of sodium tartrate, and then a solution of 0.25 g. phenylhydrazine in acetic acid. Heat the flask for 10 minutes in a boiling-water bath. Add a measured quantity of standard titanium solution, in considerable excess over that required for the reduction of the osazone, and boil for 2 minutes while passing carbon dioxide through the flask. Add excess hydrochloric acid, and titrate the still hot solution back with a solution of crystal scarlet, which has been standardized against the titanium solution, until a permanent red color is obtained. The difference between the milliliters of titanium solution added, and the milliliters of crystal scarlet solution used in the back titration, is equivalent to the glucose. Since 1 molecule of glucose, in the form of its osazone, corresponds to 6 atoms of hydrogen, or 6 atoms of iron, the glucose is calculated by multiplying the iron value of the titanium solution by 180/336, $= 0.5357$. For instance, if 15.6 ml. titanium solution, equivalent to 0.001901 g. iron per ml., were required, the grams of glucose in the 10 ml. of solution used equal $15.6 \times 0.001901 \times 0.5357, = 0.01589$ g.

Fructose, maltose, lactose, and glucosamine can be determined in the same manner, also sucrose, starch, or cellulose after complete hydrolysis and neutralization. If the sugar solutions to be analyzed are very dilute, the strength of the titanium chloride solution must be reduced and less sodium tartrate added.

CHAPTER XVI

COMBINED METHODS AND THE ANALYSIS OF SUGAR MIXTURES

In previous chapters upon polariscopic and chemical methods several instances were given of the application of certain processes to the analysis of sugar mixtures. In the present chapter the problem of determining several sugars in the presence of one another will be taken up in somewhat fuller detail.

Whenever reliable direct methods for the determination of one or more of the sugars in the presence of the others are available, it is always preferable to use them. But many times, especially in dealing with complex mixtures, indirect methods must be resorted to.

If the sum of the specific rotations, copper-reducing powers, or other properties of the different sugars in a mixture can be expressed by a sufficient number of equations, the problem of determining the percentage of each sugar in the mixture may be solved by simple algebraic analysis. By thus combining the results of several distinct methods it is possible by indirect means to make an analysis of many sugar mixtures with a fair degree of accuracy. The combinations of methods which have been proposed for this purpose are almost numberless, and only a few examples will be chosen to illustrate the general principle. The methods will be grouped for convenience under the heads: (1) combined polariscopic methods; (2) combined reduction methods; (3) combined polariscopic and reduction methods.

COMBINED POLARISCOPIC METHODS

If two sugars, A and B, exhibit a known variation in specific rotation under different conditions of polarization, then the percentages, x and y, of the two sugars may be determined by means of the following equations:

$$ax + by = 100[\alpha]_D \quad (1)$$

$$a'x + b'y = 100[\alpha]_D' \quad (2)$$

in which $[\alpha]_D$ and $[\alpha]_D'$ are the specific rotations of the mixture $A + B$, a and a' the known specific rotations of sugar A, and b and b' the known specific rotations of sugar B, under the respective conditions of (1) and

(2). By determining $[\alpha]_D$ and $[\alpha]_D'$, the percentages x and y are readily calculated.

As an example of this method of analysis the determination of glucose and fructose by polarization at 20° C. and 87° C., under the conditions previously described (p. 482), is given. If the $[\alpha]_D^{20}$ and $[\alpha]_D^{87}$ of glucose are +52.5 and of fructose —92.5 and —52.5 respectively, then the $[\alpha]_D^{20}$ and $[\alpha]_D^{87}$ of a mixture containing x per cent glucose and y per cent fructose are

$$52.5\,x - 92.5\,y = 100[\alpha]_D^{20}$$
$$52.5\,x - 52.5\,y = 100[\alpha]_D^{87}$$

By determining the $[\alpha]_D^{20}$ and $[\alpha]_D^{87}$ of the mixture the percentages of glucose and fructose are readily calculated.

Any other temperature at which the $[\alpha]_D'$ of each of the sugars is known may, of course, be taken instead of 20° C. and 87° C. The results as thus calculated are only approximate and require to be corrected for the influence of concentration.

In making this correction, it must be considered that the specific rotation of each sugar in a sugar mixture is not that at its partial concentration, but that which it would show if its concentration were equal to the total sugar concentration. This rule, first enunciated by Vosburgh,[1] has been confirmed by Browne[2] and by Zerban.[3] It is but a special case of a more general law which has been formulated by Browne as follows: The physical effects of a mixed solvent are constant only when the concentration of water and of other ingredients which make up the solvent is unchanged.

Suppose that we have to deal with a mixture containing 5 g. of glucose and 10 g. of fructose in 100 ml.; the specific rotation of 5 g. glucose in 100 ml. at 20° C. would be 52.62, but that of 15 g. glucose (equal to the total sugar concentration) in 100 ml. would be 52.89, according to Tollens's formula. Similarly, that of 10 g. fructose would be —92.13, but that of 15 g. fructose —93.60, from Vosburgh's equation. The values 52.89 and —93.60, respectively, would have to be used to correct the results obtained for the effect of concentration on the specific rotation of the constituents of the mixture.

In addition to varying the temperature, changes of condition may be accomplished by making one polarization in neutral and the other in acid solution; or one polarization in water, and the other in some other solvent; or one polarization in the absence and the other in the presence

[1] *J. Am. Chem. Soc.*, 43, 219 (1921).
[2] *Louisiana Planter*, 67, 44 (1921).
[3] *J. Assoc. Official Agr. Chem.*, 8, 384 (1924/25).

of borax or other substance; in all of which changes of condition a definite known alteration in the polarizing power of one or both sugars must be produced. Obviously, the greater the degree of this change in polarizing power, the less will be the influence of experimental errors.

COMBINED REDUCTION METHODS

If two sugars, A and B, exhibit a known variation in reducing power under different conditions of analysis, then the percentages x and y of the two sugars may be determined by means of the general equations:

$$ax + by = 100\,R \quad (1)$$
$$a'x + b'y = 100\,R' \quad (2)$$

in which R and R' are the reducing powers of the mixture $A + B$, a and a' the known reducing powers of sugar A, and b and b' the known reducing powers of sugar B, under the respective conditions of (1) and (2). By determining R and R', the percentages x and y are readily calculated.

A good example of the application of the above formulas is given by Soxhlet's[4] well-known method for determining two sugars in mixture.

A comparison of the reducing powers of different sugars upon Fehling's copper solution (Soxhlet's formula) and Sachsse's mercury solution was made by Soxhlet with the results shown in Table CXXVII.

TABLE CXXVII

RELATIVE REDUCING POWER ON FEHLING'S AND SACHSSE'S SOLUTIONS

Sugar	1 g. Sugar in 1 Per Cent Solution Reduces		Milligrams of Sugar in 1 Per Cent Solution Reduce	
	Fehling's Solution	Sachsse's Solution	100 ml. Fehling's Solution	100 ml. Sachsse's Solution
	ml.	ml.	mg.	mg.
Glucose	210.4	302.5	475.3	330.5
Fructose	194.4	449.5	514.4	222.5
Invert sugar	202.4	376.0	494.1	266.0
Galactose	196.0	226.0	510.2	442.0
Milk sugar	148.0	214.5	675.7	466.0
Milk sugar hydrolyzed	202.4	257.7	494.1	388.0
Maltose	128.4	197.6	778.8	506.0

[4] *J. prakt. Chem.*, 21, 300 (1880); König's "Untersuchung," p. 217, 1898.

The results show that the various sugars differ very decidedly in their relative reducing powers upon the two reagents, glucose, for example, reducing more Fehling's but less Sachsse's solution than fructose.

The combined influences of two sugars, *A* and *B*, in their reducing powers upon Fehling's and Sachsse's solutions may be expressed as follows:

Let x = g. of reducing sugar *A* in 100 ml. of the 1 per cent sugar solution.
Let y = g. of reducing sugar *B* in 100 ml. of the 1 per cent sugar solution.
Let a = ml. of Fehling's solution reduced by 1 g. of sugar *A* in 100 ml. of solution.
Let b = ml. of Fehling's solution reduced by 1 g. of sugar *B* in 100 ml. of solution.
Let a' = ml. of Sachsse's solution reduced by 1 g. of sugar *A* in 100 ml. of solution.
Let b' = ml. of Sachsse's solution reduced by 1 g. of sugar *B* in 100 ml. of solution.
Let F = ml. of Fehling's solution reduced by 100 ml. of sugar solution.
Let S = ml. of Sachsse's solution reduced by 100 ml. of sugar solution.

Then $$ax + by = F$$

and $$a'x + b'y = S$$

For a mixture of x per cent glucose and y per cent fructose, and taking Soxhlet's values in Table CXXVII for a, b, a', and b', the equations would be

$$210.4\,x + 194.4\,y = F$$
$$302.5\,x + 449.5\,y = S$$

By determining the values F and S of the mixture of sugars, the percentages x and y are readily calculated.

In using the above, or other combined reduction methods, the constants a, b, a', and b' should be determined empirically by the chemist for the particular sugars with which he is working.

As another example of combined reduction methods may be mentioned Kjeldahl's[5] process of determining the reducing power of the mixture of two sugars in both dilute and more concentrated solution, using respectively 15 ml. and 50 ml. of mixed Fehling's solution according to the details of his reduction method (p. 798). The relative

[5] *Z. anal. Chem.*, 35, 3445–347 (1896).

differences in the copper-reducing powers under the two conditions of analysis are not sufficiently pronounced to afford a reliable basis of calculation, however, and the method has been generally condemned.

In certain applications of the combined reduction methods for analyzing sugar mixtures the error has been made of assuming that the reducing powers of the two sugars are additive, in other words, that the reducing power of a mixture of 100 mg. each of glucose and fructose would be equal to the sum of the reducing powers of these sugars at the 100 mg. concentration. That this would not be true has been shown on p. 761 under discussion of the variability in reducing power of monosaccharides and of the law of reducing action. Additive relations would obtain only if the same amount of unreduced copper were present at all stages of the reduction. An inspection of any reducing sugar table shows that with the continued removal of copper from solution the amounts of copper reduced by succeeding equal portions of sugar become less and less. Thus in the results quoted from Allihn's work (p. 763) the first 50 mg. glucose reduced 99 mg. copper; the second 50 mg. glucose 96 mg. copper; the third 50 mg. glucose 92.7 mg. copper; the fourth 50 mg. glucose 90 mg. copper; and the fifth 50 mg. glucose 85.3 mg. copper. The sum of these is 463 mg. copper reduced by 250 mg. of glucose. If it is desired to estimate the average amounts of copper reduced by aliquots of 50 mg. glucose in multiples of the latter these would be $\frac{145}{2}$ = 97.5 mg. copper per 50 mg. glucose in a total of 100 mg. and $\frac{463.0}{5}$ = 92.6 mg. copper per 50 mg. glucose in a total of 250 mg. The same rule applies to mixtures of reducing sugars. The reducing power of a mixture of 100 mg. each of glucose and fructose is not equal to the sum of the reducing powers of these sugars at the 100-mg. concentration but to the sum of one-half the reducing powers of these sugars at the 200-mg. concentration. In the same way with a mixture of 50 mg. each of glucose, fructose, mannose, and galactose the reducing power would be equal to the sum of one-quarter the reducing powers of these sugars at the 200-mg. concentration. This principle may be further illustrated by the following example based on the reduction tables of Quisumbing and Thomas:

1. 200 mg. invert sugar (100 mg. glucose + 100 mg. fructose) reduce	372.1 mg. Cu
2. Reducing power of 100 mg. glucose	201.2 mg. Cu
Reducing power of 100 mg. fructose	185.0 mg. Cu
Total	386.2 mg. Cu
3. One-half of reducing power of 200 mg. glucose	193.0 mg. Cu
One-half of reducing power of 200 mg. fructose	180.3 mg. Cu
Total	373.3 mg. Cu

The last figure checks with the reducing power of 200 mg. invert sugar within 1.2 mg, but the second is 14.1 mg. higher.

Similarly, the reducing power of a mixture containing 50 mg. glucose and 150 mg. fructose equals one-fourth of the reducing power of 200 mg. glucose plus three-fourths of the reducing power of 200 mg. fructose, etc.

The estimation of the combined reducing power of a mixture of sugars is most easily determined by means of reducing ratios, the method of calculating which is illustrated in Table CIX (p. 793). The reducing ratios of the different sugars were found by Browne[6] to be constant with Allihn's method at concentrations above 50 mg. sugar, and the calculated combined reducing power of sugar mixtures was found to agree very closely with that actually determined, as may be seen from Table CXXVIII.

TABLE CXXVIII

GLUCOSE EQUIVALENTS OF MIXED REDUCING SUGARS BY ALLIHN'S METHOD

Sugars	Grams Sugar in 25 ml.			Total Weight of Sugars	Glucose Equivalent		Error
	1	2	3		Calculated	Found	
				g.	g.	g.	
Glucose, fructose	0.0967	0.0904	.....	0.1871	0.1794	0.1780	+0.0014
Glucose, fructose	0.0484	0.0452	.....	0.0936	0.0898	0.0906	−0.0008
Glucose, fructose	0.0461	0.1408	.....	0.1869	0.1749	0.1755	−0.0006
Glucose, fructose	0.0231	0.0704	.....	0.0935	0.0875	0.0877	−0.0002
Glucose, fructose	0.0740	0.0198	.....	0.0938	0.0921	0.0927	−0.0006
Glucose, fructose	0.1786	0.0585	.....	0.2371	0.2311	0.2294	+0.0017
Glucose, galactose	0.0893	0.0293	.....	0.1186	0.1156	0.1161	−0.0005
Glucose, galactose	0.0265	0.0960	.....	0.1225	0.1127	0.1132	−0.0005
Glucose, galactose	0.0681	0.0175	.....	0.0856	0.0780	0.0764	+0.0016
Fructose, galactose	0.0155	0.1070	.....	0.1225	0.1102	0.1097	+0.0005
Fructose, galactose	0.1853	0.0569	.....	0.2422	0.2282	0.2267	+0.0015
Fructose, arabinose	0.0927	0.0285	.....	0.1212	0.1141	0.1131	+0.0010
Fructose, arabinose	0.2162	0.0429	.....	0.2591	0.2361	0.2369	−0.0008
Galactose, xylose	0.1081	0.0215	.....	0.1296	0.1181	0.1183	−0.0002
Galactose, xylose	0.1513	0.0433	.....	0.1946	0.1934	0.1933	+0.0001
Xylose, arabinose	0.0757	0.0217	.....	0.0974	0.0967	0.0981	−0.0014
Xylose, arabinose	0.0495	0.1535	.....	0.2030	0.2070	0.2083	−0.0013
Xylose, arabinose	0.0248	0.0768	.....	0.1016	0.1035	0.1044	−0.0009
Xylose, arabinose	0.1371	0.0226	0.0609	0.2206	0.2203	0.2210	−0.0007
Glucose, arabinose, xylose	0.0646	0.0822	0.0967	0.2435	0.2270	0.2280	−0.0010

The weights in columns 1, 2, and 3 are given in the order of the respective sugars as named.
The calculated glucose equivalents of the mixtures were found by multiplying the weights of each sugar by its reducing ratio and adding together the products.

The greatest difference between the calculated glucose equivalents

[6] *J. Am. Chem. Soc.*, 28, 443 (1906).

and those determined by experiment is 0.0017 g., which is within the limits of experimental error.

Method of Jackson and Mathews for Analyzing Mixtures of Glucose and Fructose.[7] The method of Jackson and Mathews for the determination of fructose alone, by reduction of a copper carbonate reagent, has been described on p. 824. Glucose has a much smaller reducing effect than fructose under the conditions of this method, 12.4 mg. of glucose giving the same amount of copper as 1 mg. of fructose. A convenient procedure for determining these two sugars in the presence of each other is to combine the copper carbonate reduction with a determination of the total reducing sugars by another reduction method, such as for instance that of Quisumbing and Thomas, for which the reduction values of both glucose and fructose are accurately known. The amounts of both glucose and fructose may then be calculated approximately by means of the formulas given on p. 970. In this case it is more convenient to express the total reducing sugars, R and R_1, as fructose. The reducing ratio of glucose to fructose in the method of Jackson and Mathews is 0.0808[8] (i.e., $1 \div 12.4$), and in the Quisumbing and Thomas method 1.0870 (i.e., $1 \div 0.920$). If the glucose is designated by x, and the fructose by y, then

$$1.0870\,x + y = R \quad \text{(Quisumbing and Thomas)}$$

$$0.0808\,x + y = R_1 \quad \text{(Jackson and Mathews)}$$

$$\text{Glucose } (x) = \frac{R - R_1}{1.0870 - 0.0808} = \frac{R - R_1}{1.0062}$$

$$\text{Fructose } (y) = R - 1.0870\,x$$

Example. A solution containing 30 mg. fructose and 70 mg. glucose in 20 ml. gave 116 mg. copper by the Jackson and Mathews method, equivalent to 35.4 mg. fructose; another 20-ml. aliquot of the same solution, diluted to 50 ml., gave 197.3 mg. copper by the Quisumbing and Thomas method, equivalent to 106.9 mg. fructose. Calculation by the formula gives 29.6 mg. fructose and 71.0 mg. glucose.

The error due to the variations in the relative reducing power of glucose to fructose in the Quisumbing and Thomas method may be eliminated by using not the average reducing ratio 1.0870 (i.e., $1 \div 0.920$), but that which corresponds to the total reducing power (197.3 mg. copper), namely, 1.0905 (i.e., $1 \div 0.917$).

[7] *Bur. Standards J. Research*, 8, 403 (1932).

[8] This figure is given by Jackson and Mathews; the quotient of 1 divided by 12.4 is 0.080646.

For routine purposes, Jackson and Mathews recommend determination of total reducing sugars by the Lane and Eynon volumetric method. Tables have been constructed which make it possible to find the results with a minimum of calculation. They are based on the same principles as the combined reduction formulas, and take the variation of the reducing ratio into consideration. The first table gives the Lane and Eynon factors, for 25 ml. of Soxhlet solution, for mixtures of glucose and fructose in all proportions, in steps of 10 per cent of either (see Appendix, Table 35).

On the basis of this table, the relation between the factors F, the titers T, and the ratio R of fructose to total reducing sugar may be expressed by the following equation:

$$F = 119.36 + 0.0471\,T + 7.3\,R$$

Since the total reducing sugars S equal $100\,F/T$, substitution of the above expression for F yields

$$S = \frac{119.36 + 0.0471\,T + 7.3\,R}{T} \times 100$$

The apparent fructose, l, found by the copper carbonate reduction, equals the true fructose, L, plus 0.0808 times the glucose; hence

$$l = L + 0.0808\,S\,(1 - R)$$

Substituting $S \times R$ for L, we obtain

$$l = S \times R + 0.0808\,S\,(1 - R) = 0.9192\,SR + 0.0808\,S$$

or

$$S = \frac{l}{0.9192\,R + 0.0808}$$

Combination of the two equations derived for S gives

$$\frac{T \times l}{100} = (119.36 + 0.0471\,T + 7.3\,R)(0.9192\,R + 0.0808)$$

Jackson and Mathews solved this equation for varying values of R and T, and constructed a table (see Appendix, Table 36) from which the ratio R may be read directly for varying values of l and T. The method of calculation is illustrated by the following example, quoted from the paper of Jackson and Mathews:

Example. A solution of glucose and fructose gave a Lane and Eynon titer of 25.89 ml., and 20 ml. of the same solution reduced 247.3 mg. of copper from the carbonate reagent. According to Table 28 the original solution contained $100 \times 71.7/20 = 358.5$ mg. of apparent fructose in 100 ml. $T \times l/100$ is then $25.89 \times 358.5/100 = 92.8$. For this figure, and for $T = 25$,

Table 36 gives 71.5 as the ratio of fructose to total sugar. Reference to Table 35 shows that for $T = 25.89$, and $R = 71.5$, the Lane and Eynon factor is 125.7. The total sugar in 100 ml. is therefore $100 \times 125.7/25.89$, $= 485.5$ mg., and the fructose alone is 71.5 per cent of this, or 347.1 mg. The glucose equals the difference between total sugars and fructose, or 138.4 mg.

For applications of this method and certain modifications of it to the analysis of products encountered in the manufacture of fructose from Jerusalem artichokes and other plants, the chemist is referred to a subsequent article by Jackson and Mathews.[9]

Many other combinations of reduction methods may be used for specific purposes. As one example the procedure of Widdowson[10] for determining glucose in mixture with fructose or maltose may be cited. In one portion of the sample the reducing power on alkaline ferricyanide solution (p. 872) is measured, and another portion is oxidized with hypoiodite (p. 895). The percentages of the two constituents are then calculated by means of two simultaneous equations.

In many cases it is preferable not to use a combination of two polariscopic or of two reduction methods, but rather to combine one polariscopic with one reduction method, in order to take advantage of large differences in polarizing and reduction ratios. Examples of this procedure are given next.

COMBINED POLARISCOPIC AND REDUCTION METHODS

I. Analysis of Mixtures Containing Two Sugars

The first reference to a combination of polarimetric and copper-reduction methods for analyzing a mixture of two reducing sugars was probably made by Apjohn,[11] following a suggestion given him by Jellett. Shortly afterward, Dupré[12] published a similar procedure. The same principle has been used in subsequent modifications by Neubauer[13] and by others. In all the earlier methods of this class the total reducing power of the mixture was determined as glucose, fructose, or invert sugar, the percentage thus obtained being taken as the total amount, or sum, of the sugars present. In the case of two sugars, A and B, the percentages x and y of each were expressed by the formula

$$x + y = R$$

in which R was the percentage of total reducing sugar determined as

[9] *Bur. Standards J. Research*, **9**, 597 (1932).
[10] *Biochem. J.*, **25**, 803 (1931).
[11] *Transactions Royal Irish Acad.*, **24**, 587 (1869).
[12] *Chem. News*, **21**, 97 (1870).
[13] *Ber.*, **10**, 827 (1877).

glucose, fructose, or invert sugar. The results calculated by such a formula, however, have only an approximate value, as the difference in copper-reducing power of the two sugars *A* and *B* has not been taken into account.

The error last mentioned has been largely obviated in the later methods of this class through the use of reduction factors (p. 792) by means of which the copper-reducing power of a sugar can be converted into the equivalent of any other reducing sugar which is selected as a standard of comparison. For this purpose glucose is usually selected, this being the most common of the reducing sugars and the one most easily obtained in a pure condition.

It was shown on p. 793 that the different monosaccharides bear an approximately constant ratio to glucose for the same weight of reduced copper. This ratio was given for several sugars and was found by Allihn's method to be 0.915 for fructose, 0.958 for invert sugar, 0.898 for galactose, 0.983 for xylose, and 1.032 for arabinose.

General Formulas for Analysis of Sugar Mixtures. If the reducing ratio of sugar *A* to glucose is *a*, and of sugar *B* to glucose *b*, then in a mixture of *x* per cent *A* and *y* per cent *B*, the combined influence is represented by the equation:

$$ax + by = R \tag{1}$$

in which *R* is the percentage of total sugars determined as glucose.

If the relative polarizing power of sugar *A* be expressed by α and that of sugar *B* by β, then, in a mixture of *x* per cent *A* and *y* per cent *B*, the combined influence is represented by the equation:

$$\alpha x + \beta y = P \tag{2}$$

in which *P* is the polarizing power of the mixture of sugars.

By combining equations (1) and (2) we obtain:

$$x = \frac{bP - \beta R}{\alpha b - a\beta} \tag{3}$$

$$y = \frac{\alpha R - aP}{\alpha b - a\beta} \quad \text{or} \quad \frac{R - ax}{b} \tag{4}$$

When the constants *a*, *b*, α, and β are known, the percentages *x* and *y* of any two monosaccharides can be calculated very closely from the percentage of total reducing sugar, determined as glucose, and from the polarizing power of the mixture.

Applications of the Method.[14] In the following applications of the preceding formulas to special problems of analysis, the polarizations were made upon a Ventzke-scale saccharimeter using the sucrose normal weight. The relative polarizing power of a sugar under these conditions is best expressed in terms of sucrose and is found by dividing its specific rotation by the specific rotation of sucrose, or +66.5.

In making up the various mixtures the sugars were weighed in a small stoppered flask. After the requisite amount of water had been added the flask was reweighed and the percentage of each sugar in the solution calculated. After the sugars were dissolved, the solutions were allowed to stand 24 hours before beginning the analysis, in order to remove all possibility of error through mutarotation. The copper reducing power was determined by Allihn's method.

Analysis of Mixtures of Fructose and Glucose.

Reducing ratio of fructose to glucose = 0.915 = a
Reducing ratio of glucose to glucose = 1.000 = b

Polarizing ratio of fructose (20° C., 10 per cent solution, Vosburgh's formula)

$$= \frac{-92.88}{+66.5} = -1.397 = \alpha$$

Polarizing ratio of glucose (20° C., 10 per cent solution, Tollens's formula)

$$= \frac{+52.74}{+66.5} = 0.793 = \beta$$

By substituting the values for a, b, α, and β in the general equations previously given, we obtain

$$\text{Per cent fructose } (F) = \frac{0.793\,R - P}{2.123}$$

$$= 0.374\,R - 0.471\,P, \qquad \text{at } 20° \text{ C.} \qquad (1)$$

$$\text{Per cent glucose} = R - 0.915\,F \qquad (2)$$

Owing to the great susceptibility of fructose to variations in specific rotation through changes of temperature and concentration, the use of a fixed polarization factor is possible only when the analyses are made under perfectly similar conditions. The values of the polarization factor of fructose for different temperatures and concentrations are given in the following table:

[14] The applications of the method to the analysis of mixtures containing two sugars are based upon a paper by Browne, "The Analysis of Sugar Mixtures," *J. Am. Chem. Soc.*, 28, 439 (1906).

Temperature, °C.	Concentration						
	1 Per Cent	2 Per Cent	3 Per Cent	4 Per Cent	5 Per Cent	10 Per Cent	25 Per Cent
15	−1.419	−1.421	−1.424	−1.426	−1.429	−1.441	−1.475
20	−1.376	−1.378	−1.381	−1.383	−1.385	−1.397	−1.427
25	−1.333	−1.335	−1.337	−1.340	−1.342	−1.352	−1.379
30	−1.290	−1.292	−1.294	−1.296	−1.298	−1.307	−1.331

The above figures were calculated from the formulas of Vosburgh:

$$[\alpha]_D^{25} = -(88.50 + 0.150\,c - 0.00086\,c^2)$$

and

$$[\alpha]_D^t = [\alpha]_D^{25} + (0.566 + 0.0028\,c)(t - 25)$$

The variations of the polarization constant due to concentration are not so large as to affect the accuracy of the calculations appreciably, and it is usually unnecessary to correct for them. In the experiments tabulated below, 10 per cent concentration was taken as the basis, but corrections were applied for the influence of temperature. If corrections are made for concentration, it should be done in accordance with Vosburgh's rule for the specific rotation of sugar mixtures, i.e., the polarization constants for both glucose and fructose should be based on the total sugar concentration, and not on the partial concentration of either sugar.

For temperatures other than 20° C. the denominator in equation (1) for fructose becomes, at 10 per cent concentration, 2.167 at 15° C., 2.078 at 25° C., and 2.033 at 30° C.

Analyses were made of seven mixtures containing known amounts of glucose and fructose. A 10 per cent solution of the fructose used in these experiments showed a specific rotation of −90.18 at 20° C., considerably lower than that calculated from the formula of Vosburgh, but agreeing with that calculated from the formula of Jungfleisch and Grimbert. For this reason the basic value −90.18/66.5, = −1.356 at 20° C. was used as the polarization constant in the calculations. The results of the analysis are shown on the top of p. 980.

The percentage of invert sugar in mixtures of glucose and fructose is easily found by combining the smaller percentage with an equal amount of the other component. Thus, in the first experiment of the series shown there would be 1.96 per cent invert sugar and 1.13 per cent glucose, and in the last experiment 7.52 per cent invert sugar and 7.47 per cent fructose. The formulas for calculating the percentages of glucose and fructose in mixture admit of numerous applications.

Taken		R	P	Temp.	Found		Error	
Fructose	Glucose				Fructose	Glucose	Fructose	Glucose
per cent	per cent			° C.	per cent	per cent	per cent	per cent
0.99	2.06	3.01	+ 0.35	22°	0.98	2.11	−0.01	+0.05
1.59	5.92	7.41	+ 2.65	23°	1.56	5.98	−0.03	+0.06
3.17	11.83	14.54	+ 5.30	23°	3.02	11.78	−0.15	−0.05
4.52	4.84	9.06	− 2.15	22°	4.51	4.83	−0.01	−0.01
5.63	1.85	7.02	− 6.00	23°	5.61	1.89	−0.02	+0.04
9.04	9.67	17.80	− 4.30	22°	8.90	9.66	−0.14	−0.01
11.26	3.69	14.04	−12.00	23°	11.23	3.76	−0.03	+0.07
					Average error....		−0.06	±0.04

The determinations of fructose by this means have been found to show usually a very close agreement with the results obtained by the method of high-temperature polarization, when other copper-reducing or optically active substances are absent.

In the determination of fructose and glucose in cider vinegar, Mott[15] has shown that the presence of copper-reducing aldehydes may introduce a considerable error in the calculation. If the aldehydes, however, are first volatilized by evaporating the vinegar to dryness in a platinum dish, dissolving the solids in water, and again evaporating several times, the true copper-reducing power of the mixed sugars is obtained, in which case the results of the calculation agree closely with those obtained by the method of high-temperature polarization. The following table by Mott gives the percentages of fructose and glucose in the dry substance of several cider vinegars as calculated by Browne's formula and the excess of fructose over glucose as thus found and as determined by polarization at 87° C.

Variety of Vinegar	Computed by Formulas of Browne			Excess of Fructose over Glucose by Polarizing at 87° C.
	Fructose in Solids	Glucose in Solids	Excess of Fructose	
	per cent	per cent	per cent	per cent
Baldwin.....	19.7	8.8	10.9	10.9
King.....	18.7	7.4	11.3	11.8
Greening.....	23.1	9.1	14.0	13.9
Russet.....	16.0	8.6	7.4	7.2
Mixed, pressing..	14.2	7.1	7.1	8.6

Analysis of Other Sugar Mixtures. The composition of mixtures of any two reducing sugars may be ascertained in a perfectly analogous

[15] *J. Ind. Eng. Chem.*, 3, 747 (1911).

manner as for mixtures of glucose and fructose, by substituting in the formulas the appropriate constants. Those for galactose, arabinose, and xylose are as follows:

	Reducing Ratio	$[\alpha]_D^{20}$	Polarizing Ratio
Galactose	0.898	+ 80.49	1.210
Arabinose	1.032	+105.10	1.580
Xylose	0.983	+ 18.79	0.283

Hence, for a mixture of glucose and galactose, the following formulas are used:

$$\text{Per cent glucose } (G) = \frac{1.210\,R - 0.898\,P}{0.498}$$

$$= 2.430\,R - 1.803\,P, \text{ at } 20^\circ \text{ C.} \quad (3)$$

$$\text{Per cent galactose} = \frac{R - G}{0.898} \quad (4)$$

The specific rotation of galactose varies somewhat with temperature and concentration, the differences, however, being much less than those of fructose. The following values for the polarization factor of galactose at different temperatures and concentrations were calculated from the general formula of Meissl.

Temperature °C.	10 Per Cent	15 Per Cent	20 Per Cent
10	1.242	1.248	1.254
20	1.210	1.216	1.222
30	1.179	1.185	1.191

The concentration influence of galactose upon the polarization factor is too slight to influence the calculations appreciably; the temperature influence, however, should be regarded if the readings are made very much above or below 20° C.

The formulas for a mixture of fructose and galactose are

$$\text{Per cent fructose } (F) = \frac{1.210\,R - 0.898\,P}{2.362}$$

$$= 0.512\,R - 0.380\,P \text{ (at } 20^\circ \text{ C.)} \quad (5)$$

$$\text{Per cent galactose} = \frac{R - 0.915\,F}{0.898} = 1.114\,R - 1.019\,F \quad (6)$$

The susceptibility of the specific rotations of both fructose and galactose to temperature variations necessitates a considerable correc-

tion if the polarizations are made much above or below 20° C. By using the polarization factors for fructose and galactose previously given, formula (5) can be corrected for any desired temperature. Thus, for 30° C.,

$$\text{Per cent fructose} = \frac{1.179\,R - 0.898\,P}{2.253}$$

For a mixture of fructose and arabinose

$$\text{Per cent fructose } (F) = \frac{1.580\,R - 1.032\,P}{2.888}$$

$$= 0.547\,R - 0.357\,P \text{ (at 20° C.)} \qquad (7)$$

$$\text{Per cent arabinose} = \frac{R - 0.915\,F}{1.032}$$

$$= 0.969\,R - 0.887\,F \qquad (8)$$

Correction for changes in temperature is made as in the previous cases.

The formulas for a mixture of xylose and arabinose are

$$\text{Per cent xylose } (X) = \frac{1.580\,R - 1.032\,P}{1.261}$$

$$= 1.253\,R - 0.818\,P \text{ (at 20° C.)} \qquad (9)$$

$$\text{Per cent arabinose} = \frac{R - 0.983\,X}{1.032}$$

$$= 0.969\,R - 0.953\,X \qquad (10)$$

Browne used this method in the analysis of a number of mixtures of known composition. The polarizing ratios were corrected for temperature as shown on p. 979, but not for concentration. The average deviations of the results found, from the percentages of each sugar actually present, were as follows:

Mixture	Average Deviation from Theoretical
	Per Cent
Glucose	±0.17
Galactose	±0.18
Fructose	±0.07
Galactose	±0.09
Fructose	−0.02
Arabinose	−0.07
Xylose	+0.10
Arabinose	±0.05

The five special cases which have been selected are sufficient to illustrate the principle and comparative accuracy of the combined polari-

scopic and reduction methods described for analyzing mixtures of two reducing sugars. The method can also be used in analyzing mixtures which contain rhamnose, fucose, mannose, sorbose, etc.; in those cases where the reduction factors are not known, the chemist can readily determine them for the particular conditions of the reduction method he desires to use.

The degree of accuracy obtainable by a given combination of polariscopic and reduction methods is greatest, other conditions being equal, where there is the greatest difference between the specific rotations and reducing powers of the two sugars. The probable errors of the method are always indicated by the magnitude of the factors for R and P in the different equations. Thus an error in copper-reducing power is made six times as great in equation (3) as in equation (1), and an error in polarization five times as great in equation (3) as in equation (7).

Mathews's Combined Reduction and Polarization Method for Analyzing Mixtures of Glucose and Fructose. "Mathews Ratio." A more rigorous treatment of the combined polariscopic and reduction method for determining glucose and fructose in mixtures has been formulated by Mathews.[16] This takes into consideration not only Vosburgh's rule for the specific rotation of sugar mixtures, but also the variation in reducing ratios with concentration.

If the concentration, in grams per 100 ml., of fructose be designated by x, and that of glucose by y, the polarization P of any mixture of these two sugars, in saccharimetric degrees at 20° C. may be expressed by the formula:

$$P = -[5.31154 + 0.0064928(x + y)]x + [3.03537 + 0.0020837(x + y)]y$$

This is a combination, by Vosburgh's rule, of Jackson's normal-weight formula for glucose (p. 299), and of Jackson and Mathews' normal-weight formula for fructose (p. 299).

The total reducing sugar S, as found by the Lane and Eynon titration is given by the formula (see p. 975):

$$S = \frac{100 \times (119.36 + 0.0471\,T + 7.3\,R)}{T}$$

where $R = x/(x + y)$, and T is the Lane and Eynon titer.

S is related to the total reducing sugars determined saccharimetrically by the equation:

$$D \times S = x + y$$

[16] *Bur. Standards J. Research*, 8, 433 (1932).

where D is the number of volumes to which 1 volume of the solution has been diluted for the Lane and Eynon titration.

With P, T, and D known, x and y can be computed by means of the equations given above. Mathews has compiled a table (Appendix, Table 37) by which the ratio R of fructose to total reducing sugars may be found from $(P \times T)/D$. Its use may be illustrated by the following example.

Example. A solution containing fructose and glucose gave a polarization of $-43.8°$ in the saccharimeter. Five milliliters of the solution was diluted to 100 ml., and a Lane and Eynon titer of 26.18 ml. was found. Then $D = 100/5, = 20$, and $PT/D = -43.8 \times 26.18/20, = -57.3$. The ratio R corresponding to this figure, at $T = 26.18$ ml., is 89.8 per cent, but since the relationship is not exactly linear, R must be corrected by means of the expression $f \times D/T$. The value of f is found from the same table. In this particular case the correction is found to be $-0.80 \times 20/26.18$, or -0.6. The corrected Mathews ratio R is therefore $89.8 - 0.6, = 89.2$.

The total sugar concentration is found from the Lane and Eynon titration T and factor F (p. 975). It equals $100\,F/T, = 100 \times 127.0/26.18, = 485.1$ mg. in 100 ml. of the solution titrated. The concentration of the solution read in the saccharimeter was 20 times as large, $0.4851 \times 20, = 9.702$ g. in 100 ml. of the original solution. The fructose is 89.2 per cent of this, or 8.654 g. in 100 ml. The glucose is 10.8 per cent, or 1.048 g.

The combination of reducing effect and polarization may be used also for the analysis of mixtures containing the disaccharides lactose and maltose, although, as previously stated, the reducing ratios of these sugars show much larger variations that those of the monosaccharides. A reducing ratio to glucose of 0.7 for lactose hydrate, and of 0.6 for maltose, may be employed for Allihn's method with a fair degree of approximation. In a mixture of glucose and lactose, the percentage of lactose equals $4.26\,P - 3.37\,R$. However, since the polarizing ratio of lactose is practically the same as that of glucose (0.79), the experimental error is greatly multiplied.

Better results are obtained in the analysis of mixtures of glucose and maltose, especially if the variation in the reducing ratio is taken into consideration, as in the following method.

Method of Morris for Determining Glucose and Maltose in Starch-Conversion Products.[17] In this procedure it is assumed that the yeast used completely ferments the glucose and maltose present, but has no effect on the other constituents of the product. A 10 per cent solution of the product is prepared. This is diluted to five times its volume, and 10 ml. of the solution, containing 0.2 g. of sample, is used for

[17] Allen's "Commercial Organic Analysis," 5th ed., Vol. I, p. 476, 1923.

determining the reducing power by the method of Brown, Morris, and Millar (p. 799). The weight of cupric oxide[18] found, multiplied by 500, gives the amount obtained from 100 g. of sample. Another portion of the 10 per cent solution is polarized, and the specific rotation calculated. If a saccharimeter is used, $[\alpha]_D = (10 \times 0.346 \times {}^\circ V.)/2$.

Still another portion of the 10 per cent solution is completely fermented by means of yeast, and the reducing power and specific rotation are determined as before. If the difference in the amount of cupric oxide from 100 g. of original material, before and after fermentation, is called a, and the difference in the specific rotation is called b, and if the specific rotation of glucose and of maltose is taken to be 52.7 and 138.0, respectively, then

$$mD + pM = a$$
$$52.7\,D + 138\,M = 100\,b$$

where m is the weight of cupric oxide corresponding to 1 g. of glucose, from the tables of Brown, Morris, and Millar, and p the same for 1 g. of maltose.

From the above equations,

$$\text{Per cent maltose } (M) = \frac{52.7\,a - 100\,mb}{52.7\,p - 138.0\,m}$$

$$\text{Per cent glucose } (D) = \frac{a - pm}{m}$$

The glucose and maltose may also be separated from each other and from the dextrins by biochemical methods. Van Voorst[19] ferments the glucose alone with *Torula lactosa* (Kluyver), the glucose and maltose with *Saccharomyces cerevisiae*. The reducing power of the solution is determined before and after the two fermentations by means of the method of Luff-Schoorl (p. 830). The original reducing effect (1) is due to glucose, maltose, and non-fermentable reducing substances (dextrin, etc.); that after fermentation with *Torula lactosa* (2) to non-fermentable reducing substances and maltose; and that after fermentation with *Saccharomyces cerevisiae* (3) to the non-fermentable reducing substances alone. Hence (1) minus (2) gives a measure of the glucose, and (2) minus (3) a measure of the maltose.

Somogyi[20] has found that a large excess of washed baker's yeast is capable of fermenting glucose at a *p*H of 7.2 to 8 in a short period of time, while maltose is not attacked under these conditions. Glucose

[18] The method of using weights of cupric oxide instead of corresponding weights of sugar is faulty for the reason explained on p. 972.

[19] *Chem. Weekblad*, 35, 338 (1938).

[20] *J. Biol. Chem.*, 119, 741 (1937).

and maltose may thus be determined in mixtures with dextrins by the following procedure:

Measure 15 ml. of a suspension of 10 g. washed baker's yeast in 100 ml. of water into a Pyrex test tube, 150 by 16 mm.; centrifuge, decant, drain off the supernatant water, and dry the wall of the test tube with a strip of filter paper. Add 15 ml. of the sugar solution, which should contain not more than 40 mg. sugars in 100 ml., and immediately make alkaline by adding 1 ml. of a 1.6 per cent solution of sodium carbonate. Stir up the yeast with a glass rod and allow to ferment for 30 minutes at a temperature not below 25° C. A drop of phenol red indicator should be added, and, if the red color fades during the fermentation, the alkalinity must be restored by adding a little sodium carbonate solution. During the fermentation the tube should be inverted from time to time to prevent the yeast from settling. At the end of 30 minutes the tube is centrifuged and the supernatant solution decanted. Five-milliliter portions of the original solution and of the fermented solution are pipetted out, and the reducing power is determined by the micromethod of Shaffer and Somogyi (p. 846), with the following reagent, prepared like the Somogyi reagent described on p. 848, but containing in 1 liter 25 g. anhydrous sodium carbonate, 25 g. Rochelle salt, 40 ml. 0.1 *N* sodium hydroxide solution, 6 g. crystallized copper sulfate, 5 g. potassium iodide, 200 g. anhydrous sodium sulfate, and 15 ml. of 0.1 *N* potassium iodate solution. This solution is standardized with pure glucose and pure maltose, with a heating period of 15 minutes. The difference between the reducing effect of the original solution and that after fermentation in alkaline solution represents the glucose in the mixture. The residual reduction represents the maltose if no other reducing substances are present. Otherwise a portion of the solution is fermented for 2 to 2½ hours without the addition of alkali. In this case the maltose is fermented also, and the reducing power still remaining is due to non-fermentable reducing substances.

II. Analysis of Mixtures Containing Three Sugars

The indirect method of combining polarization and reducing power can also be applied, but with considerable limitations, to the analysis of mixtures containing three sugars.

Methods Based upon a Determination of Total Sugars, Reducing Power, and Polarization. The calculation of three sugars in a mixture is sometimes made: (1) from a determination of the total sugars, as by drying or by densimetric means; (2) from the reducing power; and (3) from the polarization.

If three sugars A, B, and C constitute a mixture, and no other substances are present, the percentages x, y, and z of each may be expressed as follows:

$$x + y + z = T \text{ (total solids)} \quad (1)$$
$$ax + by + gz = R \text{ (reducing sugars as glucose)} \quad (2)$$
$$\alpha x + \beta y + \gamma z = P \text{ (polarization)} \quad (3)$$

T, R, and P having been determined, and the reducing constants a, b, and g and polarizing constants α, β, and γ of the three sugars being known, the percentages x, y, and z of each may be calculated in certain cases with a fair degree of approximation. It frequently happens, however, in making calculations by this method that small experimental errors are enormously multiplied, so that the final results, even with mixtures of pure sugars, can be regarded as only very roughly approximate.

Analysis of a Mixture Containing Glucose, Galactose, and Fructose. As an example of the limitations above mentioned, the problem of analyzing a mixture containing x per cent glucose, y per cent galactose, and z per cent fructose is taken. By substituting the reducing and polarizing constants previously employed for these three sugars in the general equations (1), (2), and (3) we obtain:

$$x + y + z = T$$
$$x + 0.898\,y + 0.915\,z = R$$
$$0.793\,x + 1.210\,y - 1.397\,z = P, \quad \text{at } 20^\circ \text{ C.}$$

whence

$$z = \text{per cent fructose} = 1.924\,T - 1.611\,R - 0.394\,P, \quad \text{at } 20^\circ \text{ C.} \quad (1)$$
$$y = \text{per cent galactose} = 8.200\,T - 8.460\,R + 0.328\,P, \quad \text{at } 20^\circ \text{ C.} \quad (2)$$
$$x = \text{per cent glucose} = T - y - z \quad (3)$$

It is seen that any experimental errors in determining total solids or reducing sugars are magnified in the calculation of galactose over eight times.

Example. A solution containing 6.46 per cent glucose, 8.22 per cent galactose, and 9.67 per cent fructose ($[\alpha]_D^{20} = -90.18$; polarization ratio = 1.356) gave upon analysis the following results: Total solids (T) by drying in vacuo 24.20 per cent; reducing sugars (R) as glucose 22.80 per cent; polarization (P for 26 g. in 100 ml., 200-mm. tube at 20° C.) +1.95° V. Substituting these values for T, R, and P in the previous equations gives fructose 9.23 per cent; galactose, 6.21 per cent; and glucose, 8.76 per cent.

The relationships between experimental errors and the errors in calculated results in the previous example are as follows:

	Theoretical	Found	Error	
Total solids	24.35	24.20	−0.15	Experimental
Reducing sugars as glucose	22.70	22.80	+0.10	Experimental
Polarization	+1.96	+1.95	−0.01	Experimental
Glucose	6.46	8.76	+2.30	Calculated
Galactose	8.22	6.21	−2.01	Calculated
Fructose	9.67	9.23	−0.44	Calculated

It is seen that a combination of very slight experimental errors introduces an error of more than 2 per cent in the calculation of glucose and galactose.

Analysis of a Mixture Containing Glucose, Fructose, and Sucrose. When one of the three sugars in a mixture is non-reducing, the calculation by the above indirect method can frequently be made with a much greater degree of accuracy. Thus, for a mixture containing x per cent glucose, y per cent fructose, and z per cent sucrose, the three general equations would give:

$$x + y + z = T$$
$$x + 0.915\,y = R$$
$$0.793\,x - 1.397\,y + z = P, \quad \text{at } 20^\circ \text{ C.}$$

whence

$$y = \text{per cent fructose} = 0.453\,(T - P) - 0.094\,R, \quad \text{at } 20^\circ \text{ C.} \tag{4}$$
$$x = \text{per cent glucose} = R - 0.915\,y \tag{5}$$
$$z = \text{per cent sucrose} = T - x - y \tag{6}$$

It is seen that in a mixture of glucose, fructose, and sucrose there is a division, rather than a multiplication, of experimental errors in the calculation.

Example. A solution containing 5.43 per cent fructose ($[\alpha]_D^{20} = -90.18$; polarization ratio = 1.356), 10.02 per cent glucose, and 16.16 per cent sucrose gave upon analysis the following results: Total solids (T) by drying in vacuo 31.50 per cent; reducing sugars (R) as glucose by Allihn's method, 15.24 per cent; polarization (P, 26 g. in 100 ml., 200-mm. tube at 25° C.) +17.05° V. Substituting these values for T, R, and P in equations (4), (5), and (6) gives 5.40 per cent fructose, 10.30 per cent glucose, and 15.80 per cent sucrose.

The relationship between experimental errors and the errors in calculated results in the above example are as follows:

	Theoretical	Found	Error	
Total solids	31.61	31.50	−0.11	Experimental
Reducing sugar as glucose	14.99	15.24	+0.25	Experimental
Polarization	+16.75 (20° C.)	+17.05 (25° C.)	+0.30	Experimental
Fructose	5.43	5.20	−0.23	Calculated
Glucose	10.02	10.48	+0.46	Calculated
Sucrose	16.16	15.82	−0.34	Calculated

It is seen that the calculation by this method gives a very good approximation, notwithstanding the influence of rather large experimental errors (due to polarizing at 25° C. instead of 20° C. and to the slight reducing action of sucrose).

Analysis of a Mixture Containing Glucose, Maltose, and Dextrin. Several indirect methods based upon determinations of total solids, reducing power, and polarization have been proposed for the analysis of starch-conversion products which contain the three carbohydrates, glucose, maltose, and dextrin.

In the method proposed by Allen[21] the $[\alpha]_D$ of glucose is taken as +52.7, of maltose as +139.2, and of dextrin as +198.0. The copper-reducing power of glucose is taken as 1, of maltose as 0.62, and of dextrin as 0. The sum of the glucose (g), maltose (m), and dextrin (d) is taken as the total organic solids (O), and is found by subtracting the percentage of ash from the percentage of total dry substance. The three general equations used by Allen are:

$$g + m + d = O \text{ (organic solids)}$$
$$g + 0.62\,m = K \text{ (copper-reducing power by O'Sullivan's method)}$$
$$52.7\,g + 139.2\,m + 198\,d = 100\,S \text{ (specific rotation)}$$

By substituting the first equation in the last and transposing we obtain:

$$139.2\,m = 100\,S - 52.7\,g - 198\,(O - g - m)$$

By substituting $K - 0.62\,m$ for g in the preceding equation and transposing, we obtain:

$$31.3\,m = 100\,S - 52.7\,K - 198\,(O - K)$$

Dividing the above by 100 we obtain:

$$m = \left(S - \frac{52.7\,K + 198\,(O - K)}{100}\right) \div 0.313$$
$$= 3.195\,S + 4.642\,K - 6.326\,O \quad (1)$$
$$g = K - 0.62\,m \quad (2)$$
$$d = O - g - m \quad (3)$$

[21] Allen's "Commercial Organic Analysis," Vol. I, 365, 1901.

If the sample is polarized upon a saccharimeter, and a reading P is obtained with a normal weight solution, formula (1) becomes

$$m = 2.125\,P + 4.642\,K - 6.326\,O$$

The total solids T are preferably determined by drying on sand in vacuo. If the method of solution factors (p. 54) is used, corrections must be applied for differences in the solution factors of commercial glucose (3.86) and of its ingredients, viz., 3.83 for glucose (g), 3.92 for maltose (m), 4.21 for dextrin (d),[22] and 8 for ash (a),[23] respectively. Then

$$O = T_{3.86} - \frac{3.86\,a}{8}$$

where $T_{3.86}$ is the total solids as calculated from the specific gravity by the solution factor 3.86. In Allen's equations for O, K, and 100 S, g must be multiplied by the factor 3.86/3.83, m by 3.86/3.92, and d by 3.86/4.21.

Several other methods of calculating maltose, glucose, and dextrin, similar to that of Allen, have been proposed, slightly different values being used for the polarizing and reducing constants.

It is seen that in the calculation of maltose by Allen's method any experimental errors in determining organic solids, reducing power, or specific rotation are greatly multiplied. The value of the method in the analysis of hydrolyzed starch products is still further diminished by the fact that no account is taken of isomaltose and of the various reversion products which are always present in materials of high conversion. Any reducing power and rotation due to other substances than glucose, maltose, and dextrin affect the accuracy of the method to a marked degree. Furthermore the dextrins of starch conversion are of a mixed character with different rotations and reducing powers, so that the selection of an initial dextrin of $[\alpha]_D$ +198 and zero reducing power is largely arbitrary. The percentages of glucose, maltose, and dextrin in starch-conversion products, as calculated from determinations of organic solids, reducing power, and polarization, are, therefore,

[22] It is noted that the solution factors of glucose, maltose, and dextrin increase in the order of their specific rotations. From this relationship Rolfe (*J. Am. Chem. Soc.*, 19, 698) has derived a general equation $\Sigma = 0.004023 - 0.000001329\,(195 - [\alpha]_D)$, for calculating the specific gravity influence of any acid-hydrolyzed starch solution, when the value for $[\alpha]_D$ (obtained by the factor 0.00386 between the densities 1.035 and 1.045) is known. The value for Σ multiplied by 1000 will give of course the O'Sullivan solution factor.

[23] Allen's "Commercial Organic Analysis," Vol. I, p. 376, 1901.

largely conventional quantities; the latter, when properly understood, however, may serve as a valuable means of comparison.

The methods of estimating three sugars in mixture which depend upon a determination of total sugars become largely valueless for such products as molasses, fruit juices, honeys, etc., which contain varying amounts of organic and mineral salts, gums, and acids. With such materials a determination of dry substance or of organic solids gives too high a percentage of total sugars, and the results of the calculation may even lack the value of an approximation. It is, therefore, always the best plan to determine as many of the sugars as possible in a mixture by direct means.

Methods of Calculating the Percentages of Three Sugars from the Combined Reducing Power and Polarization and the Direct Determination of One Sugar. If, in a mixture of three sugars containing x per cent A, y per cent B, and z per cent C, the percentage z of C is determined by direct means, then x and y can be calculated by means of the following equations:

$$ax + by + gz = R \text{ (total reducing sugars as glucose)}$$
$$\alpha x + \beta y + \gamma z = P \text{ (polarization)}$$
$$z = Z \text{ (direct determination)}$$

whence

$$ax + by = R - gZ$$
$$\alpha x + \beta y = P - \gamma Z$$

R, P, and Z having been determined, and the reducing and polarizing constants of the three sugars being known, the percentages x and y can be calculated as described on p. 977, for mixtures of two sugars.

Several applications of the method will be described.

Analysis of a Mixture Containing Glucose, Fructose, and Sucrose. The sucrose is best determined by the methods of inversion, using either the process of double polarization or that of copper reduction. If the polariscopic method is used, the inversion is best accomplished by means of invertase in order to eliminate the influence of the acid upon the rotation of fructose.

If the percentage (S) of sucrose is known in a mixture containing x per cent glucose and y per cent fructose, and no other optically active or reducing substances, the percentages x and y can be calculated by means of the two equations:

$$x + 0.915\,y = R \text{ (reducing sugars as glucose)}$$

$$0.793\,x - 1.397\,y + S = P, \text{ 20° C.} \left(\begin{array}{c}\text{polarization of a sucrose normal}\\ \text{weight on a saccharimeter}\end{array}\right)$$

whence

$$y = \text{per cent fructose} = \frac{0.793\,R + S - P}{2.123}, \quad \text{at } 20^\circ \text{ C.} \qquad (1)$$

$$x = \text{per cent glucose} = R - 0.915\,y \qquad (2)$$

The determination of R will be a little too high, unless a correction is made for the slight reducing action of sucrose upon Fehling's solution. This correction can be made by using an empirical formula, such as proposed by Browne for Allihn's method (p. 805), or by using the special methods and tables for determining reducing sugars in the presence of sucrose.

Example. The solution employed in the previous example (p. 988) gave by the method of inversion 16.27 per cent of sucrose (S); substituting this and the previous values, $R = 15.24$, and $P = +17.05$ at 25° C., in equation (1), and changing the denominator from 2.123 to 2.08 in order to make allowance for the polarization constant 1.356 of the fructose used, we obtain:

$$\text{Fructose} = \frac{0.793\ (15.24) + 16.27 - 17.05}{2.08} = 5.43 \text{ per cent}$$

$$\text{Glucose} = 15.24 - 0.915(5.43) = 10.27 \text{ per cent}$$

These percentages agree more closely than in the previous example with the actual amounts of sugars taken, viz.: 5.43 per cent fructose, 10.02 per cent glucose, and 16.16 per cent sucrose.

MISCELLANEOUS METHODS FOR ANALYZING MIXTURES OF THREE SUGARS

While the method just described gives reliable results with mixtures containing only these three sugars, its usefulness in the analysis of molasses and similar products is greatly reduced by the fact that the direct polarization of these materials is usually affected by the presence of other optically active substances. Besides, the lead subacetate generally used for clarification of solutions prior to polarization has a decided effect on the rotation of some of the substances present, notably fructose, asparagine, glutamine, aspartic acid, and glutaminic acid. For this reason the value of P in the above formula must not be the direct polarization found in the presence of the excess lead, but that obtained after careful deleading, as is practiced in the invertase method of the Association of Official Agricultural Chemists (p. 432). In the analysis of raw cane sugars the difference between S and P is usually so small that the permissible deviations may cause large errors in the ratio between glucose and fructose.

It is therefore preferable in the analysis of these products to use combined reduction methods rather than a combination of a polariscopic with a reduction method, for determining glucose and fructose.

Method of Jackson, Mathews, and Chase for Mixtures of Glucose, Fructose, and Sucrose.[24] The sucrose, as in the method described previously, is determined directly by inversion with invertase. Then either of the following two procedures may be employed:

(*a*) The inverted solution is diluted to a suitable concentration, and glucose and fructose are estimated by a combination of the Lane and Eynon volumetric and the modified Nijns method, as described on p. 975. Both glucose and fructose are corrected for the glucose and fructose formed in the inversion of the sucrose, by deducting from each 1.053 (i.e., $\frac{8}{8}\frac{5}{4}\frac{0}{2}$) times one-half of the sucrose equivalent.

(*b*) The original solution, containing the sucrose, is diluted and analyzed for glucose and fructose by a combination of the Lane and Eynon volumetric and the modified Nijns method, and the results of both methods are corrected for the reducing effect of the sucrose. The corrections to be applied to the milligrams of copper found by the modified Nijns method are found on p. 824. With the Lane and Eynon method the corrections must be determined experimentally. If the ratio of sucrose to total reducing sugars is very low, as for instance in honeys and many fruit juices, the correction for the reducing effect of sucrose is so small as to be negligible. Jackson, Mathews, and Chase have used both modifications (*a*) and (*b*) for the analysis of such materials (see p. 1009).

If, on the other hand, the ratio between sucrose and total reducing sugars is very high, as in raw sugars, procedure (*a*) would lead to considerable error. In this case it is necessary to use procedure (*b*), with corrections for the reducing effect of the sucrose.

Method of Zerban and Wiley for Determining Glucose and Fructose in Raw Sugars.[25] The corrections just referred to are made by means of the Lane and Eynon factors established by Zerban and Wiley for glucose and for fructose in the presence of 10 or 25 g. of sucrose in 100 ml. solution, titrated against 10 ml. of Soxhlet solution. The sucrose having been determined by means of the double polarization method with invertase, the glucose x and fructose y are calculated by the formulas given below, in which R represents the milligrams of total reducing sugars, expressed as fructose and corrected for the reducing effect of the sucrose, by the Lane and Eynon titration; a the variable reducing ratio of glucose to fructose, in the same method; R_1 the milli-

[24] *Bur. Standards J. Research*, 9, 597 (1932).

[25] *Ind. Eng. Chem., Anal. Ed.*, 6, 354 (1934); 8, 321 (1936).

grams of apparent fructose, corrected for the reducing effect of the sucrose, by the modified Nijns method of Jackson and Mathews, in the same volume of solution as was used for determining R; and 0.0806 represents the constant reducing ratio of glucose to fructose in the modified Nijns method.

$$\begin{aligned} ax + y &= R \\ 0.0806\,x + y &= R_1 \\ \hline (a - 0.0806)\,x &= R - R_1 \end{aligned}$$

$$\text{Glucose } (x) = \frac{R - R_1}{a - 0.0806}$$

$$\text{Fructose } (y) = R - ax$$

Table 38 in the Appendix gives the Lane and Eynon factors for fructose in the presence of 10 and 25 g. of sucrose, respectively, the values of a, and also those for a — 0.0806, for Lane and Eynon titers from 15 to 50 ml. The chemist should always check the Lane and Eynon factors and the values of a, or establish his own figures under the exact conditions used by him.

The details of the method are as follows:

A solution of the raw sugar, containing 25 g. of sucrose in each 100 ml., is prepared (the direct polarization may be taken to equal the sucrose in most cases without serious error). A total of 250 ml. of solution is usually sufficient. The solution is clarified with neutral lead acetate solution before being made up to the mark. It is filtered, deleaded with dry potassium oxalate, and refiltered. Two 20-ml. portions of the final solution are used to determine in duplicate the reducing effect on the copper carbonate solution, according to Jackson and Mathews. The copper in the precipitate is determined volumetrically by the ferric sulfate and permanganate method (see p. 776), since weighing usually gives too high results because of contamination; electrolytic reduction, or the chromic acid-ferrous sulfate method of Jackson and Mathews, may also be used. The remainder of the filtrate is used for the Lane and Eynon titrations, and the factor is found from Table 38B in the Appendix. If the titer is less than 15 ml., 100 ml. of the clarified solution is diluted to 250 ml., the new solution now containing 10 g. of sucrose in 100 ml., and the factor is found from Table 38A in the Appendix. If, on the other hand, the titer of the original clarified solution is over 50 ml., a new solution of the raw sugar is prepared and a known quantity of fructose or invert sugar is added, as suggested for such cases by Eynon and Lane. The added reducing sugar is later corrected for.

Example. A raw sugar was found to contain 96.75 per cent sucrose. Therefore a solution was prepared containing 25.84 g. raw sugar or 25.00 g. sucrose in each 100 ml. The Lane and Eynon titer was found to be 18.13 ml. Accord-

ing to Table 38B, this corresponds to 44.3 × 100/18.13, = 244.3 mg. total reducing sugars, expressed as fructose, in 100 ml. solution.

By the Jackson and Mathews method 76.9 mg. of reduced copper was found. Corrected for the reducing effect of the sucrose (5 g.), this gives 76.9 − 9.0, = 67.9 mg. copper. According to the table of Jackson and Mathews this corresponds to 22.0 mg. of apparent fructose in 20 ml. of solution, or 110.0 mg. in 100 ml.

Table 38B gives 0.9548 as the value of a − 0.0806, and the glucose equals (244.3 − 110.0) ÷ 0.9548, = 140.7 mg. The fructose equals 244.3 − 140.7 × 1.0356, = 98.6 mg. This gives 0.54 per cent glucose, and 0.38 per cent fructose in the raw sugar.

There is a slight error in the result, because the reducing power of glucose with respect to that of fructose varies slightly with the ratio between the two, but the maximum divergence is 0.23 per cent of the total, which is well within the limits of error of the method.

A similar method, for the determination of glucose and fructose in cane molasses, has been devised by Erb and Zerban.[26] It is a combination of the Munson and Walker method for the total reducing sugars and of the Jackson and Mathews method for the apparent fructose. It requires the use of a Munson and Walker table for glucose and for fructose alone in the presence of sucrose. For this table, and for the method of calculation, the chemist is referred to the original article.

Kruisheer's Method for Analyzing Mixtures of Sucrose, Glucose, and Fructose.[27] Kruisheer has applied the procedure of Kolthoff-Kruisheer (p. 902) for the selective determination of fructose also to the analysis of mixtures of this sugar with sucrose and glucose, such as fruit juices. The unoxidized fructose as well as the total reducing sugars are estimated by the modified method of Luff-Schoorl (p. 832), in which the reducing power of fructose is equal to that of glucose. The glucose is therefore found simply by deducting the fructose from the total reducing sugars R_1. A portion of the original solution is inverted by heating 50 ml. with 5 ml. of 30 per cent hydrochloric acid in a 100-ml. flask to 68–70° C. for 10 minutes. The solution is cooled, neutralized, cooled again, and the volume is completed to the mark. The total reducing sugars (R_2) are determined in this solution, and the value of R_1 is deducted from the result. The difference, multiplied by 0.95, gives the sucrose originally present. More exact results are obtained if invertase is used for the inversion, instead of acid.

This method, though simpler, is not so accurate as the other methods just described, in which the modified Nijns method is used. In the

[26] *Ind. Eng. Chem., Anal. Ed.*, **10, 246 (1938).**
[27] *Z. Untersuch. Lebensm.*, **58, 261 (1929).**

first place, no correction is applied for the reducing effect of the sucrose. Secondly, there is usually a slight oxidation of the fructose by the hypoiodite, while the glucose is not always completely oxidized. The last two errors are mutually compensating, but the final result may be either high or low, depending on the exact conditions of the oxidation.

Spengler's Method for Analyzing Mixtures of Sucrose, Raffinose, and Invert Sugar. It has been pointed out on p. 461 that when a beet molasses contains, besides sucrose and raffinose, also appreciable quantities of other optically active substances, as for instance invert sugar, the usual double polarization method is not applicable, and it becomes necessary to make a third, independent determination. Such a method was first employed by Baumann[28] and has been modified by Spengler.[29] The invert sugar is estimated directly, and after complete inversion with acid the reducing power and the polarization are measured.

For the determination of the reducing power before and after inversion the method of Meissl (p. 796) is used, with 50 ml. sugar solution, 50 ml. mixed Soxhlet's solution, 2 minutes' boiling, and addition of 100 ml. recently boiled and cooled water after the reduction. The invert sugar originally present is calculated by means of the Meissl and Hiller factors (Table CXII, p. 811). The increase in the reducing power after inversion is due to the invert sugar formed from the sucrose and the mixture of reducing sugars formed from the raffinose. The milligrams of copper found after inversion are converted into sucrose by multiplying the milligrams of invert sugar from Meissl's table (Appendix, Table 17) by 0.95; they are also converted into the corresponding milligrams raffinose by means of Table CXXIX (p. 997).

In the formulas given below, the ratio between milligrams of copper and milligrams of sucrose is designated by F_1, and that between milligrams of copper and milligrams of raffinose by F_2. If a grams of a beet molasses is dissolved to 50 ml., and if it contains x per cent of sucrose including that originally present as invert sugar, and y per cent of raffinose, then

$$\text{Cu} = \text{milligrams copper} = \frac{axF_1}{100} + \frac{ayF_2}{100} \qquad (1)$$

The polarization of an inverted solution of the same molasses is also determined, and the reading referred to the normal weight (P'). Since the polarization of the sucrose after acid inversion is $-33.00°$ V., and that of the raffinose after inversion is $+185.2 \times 0.5142, = 95.23°$ V.,

[28] *Z. Ver. deut. Zucker-Ind.*, **48**, 779 (1898).

[29] Frühling-Spengler, "Anleitung zu Untersuchungen in der Zuckerindustrie," p. 289 ff, 1932.

TABLE CXXIX

MILLIGRAMS OF COPPER CORRESPONDING TO MILLIGRAMS OF ANHYDROUS RAFFINOSE, AFTER INVERSION (Spengler)

Copper	Anhydrous Raffinose	F_2	Copper	Anhydrous Raffinose	F_2
mg.	mg.		mg.	mg.	
154.0	110	1.401	271.8	200	1.359
167.3	120	1.394	284.8	210	1.356
180.4	130	1.388	297.7	220	1.353
193.5	140	1.382	310.7	230	1.351
206.6	150	1.377	323.8	240	1.349
219.5	160	1.372	336.5	250	1.346
232.6	170	1.368	349.4	260	1.344
245.7	180	1.365	362.3	270	1.342
258.8	190	1.362	.....	...	.....

the following formula applies, assuming that the polarizations are additive:

$$P' = -0.3300\,x + 0.9523\,y \tag{2}$$

By combining formulas (1) and (2), and solving for x and y, we obtain

$$x = \frac{95.23\ \mathrm{Cu} - aF_2P'}{a(0.9523\,F_1 + 0.33\,F_2)} \tag{3}$$

$$y = \frac{P' + 0.33\,x}{0.9523} \tag{4}$$

The details of the method are best illustrated by an example:

Thirteen grams of a molasses was inverted with acid and made up to 100 ml. The polariscopic reading at 20° C. was −4.25° V., or −8.5° for the normal weight.

One and a quarter milliliter of the inverted solution, containing 162.5 mg. of molasses, neutralized and diluted to 50 ml., gave 184 mg. copper. According to Meissl's table $F_1 = 184/92.4 = 1.991$ (97.3 mg. invert sugar is equivalent to 92.4 mg. sucrose). According to Table CXXIX, $F_2 = 184/132.75 = 1.386$. Substitution of the values found in formula (3) gives 50.8 per cent total sucrose, and formula (4) gives 8.7 per cent raffinose.

The total sucrose must still be corrected for that equivalent to the original invert sugar.

Two grams of the original molasses in 50 ml. of solution gave 250 mg. of copper. This corresponds to approximately 125 mg. of invert sugar, or 6.25 per cent. According to Spengler, the figure 50.8, found above for the per cent total sucrose, may be used to correct the invert sugar by means of the Meissl and Hiller factors. This is obviously not quite correct, since the total sucrose

includes that corresponding to the invert sugar in the original molasses, while the possible effect of the raffinose on the reducing power of the invert sugar is left out of consideration. But applying the correction suggested by Spengler, the per cent invert sugar is found to be 6.57.

Deducting from the per cent total sucrose (50.8) that equivalent to the original invert sugar ($6.57 \times 0.95 = 6.2$) the final result of the analysis is 44.6 per cent sucrose, 8.7 per cent raffinose, and 6.6 per cent invert sugar.

The effect of raffinose on the reducing power of invert sugar should be studied and the method of calculation modified accordingly.

Analysis of a Mixture Containing Glucose, Maltose, and Dextrin. In addition to the methods previously described, several processes have been devised for determining glucose, maltose, and dextrin in starch-conversion products, which are based upon a direct determination of the dextrin.

Determination of Dextrin. Several methods have been proposed for the direct estimation of dextrin in presence of other carbohydrates. Even though some of these give reproducible results their reliability is still very much in doubt, because of the complex nature of the products.

The dextrin is sometimes precipitated from the sirupy solution by adding a large excess of hot 95 per cent alcohol, and stirring, after which the precipitate of dextrin is allowed to subside. The clear solution when deposition is complete is decanted through a filter; the dextrin is dissolved in a little water and again precipitated by adding alcohol as before. The process is repeated for a third time, after which the precipitate is washed into a platinum evaporating dish and dried and weighed. The residue is then ignited and the weight of ash deducted from the weight of dried alcohol precipitate; the difference is estimated as dextrin. The difficulty with this method of estimation is to precipitate all the dextrin without occluding any of the glucose or maltose.

The dextrin after repeated precipitations with alcohol still reduces Fehling's solution; this may be due, however, to the presence of reducing maltodextrins as well as to the occlusion of sugars.

Methods based upon a destruction of reducing sugars by fermentation or oxidation, and then calculating the residual polarizing power to dextrin, have already been referred to (p. 487). The principal objection to the fermentation method is that most yeasts ferment or modify dextrin to a greater or less degree so that the residual polarizing power does not represent that of the dextrins originally present. In Wiley's method (p. 495) of destroying reducing sugars by oxidation with alkaline mercuric cyanide, it has been found that the polarizing power of maltose is not completely destroyed and that the dextrins themselves undergo partial oxidation to dextrinic acid.

Owing to the limitations of the methods just described it is evident that the percentages of dextrin thus determined have only a nominal value.

Method of Wiley. Assuming that the residual polarizing power (P'), after destroying maltose and glucose, is due to an unchanged dextrin (d) of $[\alpha]_D$ +193, and calling the $[\alpha]_D$ of glucose (g) +53 and of maltose (m) +138, and supposing the relative reducing powers of glucose and maltose to be 100 and 62[30] respectively, the calculation of the percentages g, m, and d in a starch-conversion product is made by Wiley[31] as follows:

$$g + 0.62\,m = R \text{ (total reducing sugars as glucose)} \quad (1)$$

$$53\,g + 138\,m + 193\,d = 100\,P \quad (P = [\alpha]_D \text{ of product}) \quad (2)$$

$$193\,d = 100\,P' \quad (P' = [\alpha]_D \text{ after destroying } g \text{ and } m) \quad (3)$$

Subtracting (3) from (2) gives

$$53\,g + 138\,m = 100\,(P - P') \quad (4)$$

Multiplying (1) by 53 and subtracting from (4) gives

$$105.14\,m = 100\,(P - P') - 53\,R \quad (5)$$

whence

$$m = \frac{100\,(P - P') - 53\,R}{105.14} = 0.951\,(P - P') - 0.504\,R \quad (6)$$

$$g = R - 0.62\,m \quad (7)$$

$$d = \frac{100\,P'}{193} \quad (8)$$

Example. A sample of midzu ame (Japanese glucose) was analyzed by Wiley with the following results:

$[\alpha]_D$ before fermentation = +132.6 = P

$[\alpha]_D$ after fermentation = +59.2 = P'

Total reducing sugars as glucose = 33.33 per cent = R

Substituting these values in equations (6), (7), and (8) gives:

$$\text{Maltose} = 0.951(132.6 - 59.2) - 0.504(33.33) = 53.01 \text{ per cent}$$

$$\text{Glucose} = 33.33 - 0.62(53.01) = 0.47 \text{ per cent}$$

$$\text{Dextrin} = \frac{100\ (59.2)}{193} = 30.67 \text{ per cent}$$

[30] The ratio 62, or in decimal form 0.62, is strictly true only for O'Sullivan's method. The factor is less than this for other processes of copper reduction.

[31] Wiley's "Agricultural Analysis," Vol. III, p. 228, 1897.

If the sample is polarized upon a saccharimeter the factor for the scale readings, P and P', of a sucrose normal weight would be for equation (6)

$$100 : 66.5 :: 0.951 : x; \quad x = 0.632$$

Equation (6) of Wiley modified for the polarizations of a sucrose normal weight upon a saccharimeter would then be

$$m = 0.632\,(P - P') - 0.504\,R$$

Equation (8) of Wiley modified for calculating dextrin from the saccharimeter reading (P') of a sucrose normal weight would be

$$\frac{193}{66.5}\,d = P', \quad \text{whence } d = \frac{P'}{2.902}$$

The criticisms on p. 991 of the indirect method of estimating glucose, maltose, and dextrin from organic solids, polarization, and reducing power apply also to the method of calculation just described. Owing to the mixed character of the dextrins in starch-conversion products, the selection of a dextrin of $[\alpha]_D = +193$, or of any other fixed value, as a basis of calculation is largely conventional. The presence of the unfermentable reducing sugar isomaltose and of optically active reversion products also affects the accuracy of the method. Owing to these reasons, as well as to the general unreliability of the methods for estimating dextrin, the results of such calculations have frequently no absolute scientific value.

The difficulty due to the uncertainty in the polarizing power of the dextrin is avoided in the following method, in which the glucose and maltose are removed by fermentation, but the dextrin is found by drying after fermentation.

Method of Bryant and Jones.[32] The commercial glucose or other starch-conversion product is first analyzed for ash and protein. The total reducing sugars are determined by the volumetric Soxhlet method, and expressed as glucose.

For the fermentation experiments, the product is diluted to about 10° Brix. The ash, protein, and reducing sugars in 100 ml. of this solution are computed from the above analyses and the dilution. The dry substance is determined by drying on sand.

Portions of 500 to 600 ml. each of the solution are inoculated with 5 g. each of thoroughly washed fresh baker's yeast, and the weight and volume of the mixture are noted. The fermentation is allowed to proceed at about 80° F., until there is no further loss of weight in 24 hours. This requires about 6 to 10 days. A little "Arcady," a preparation

[32] *Ind. Eng. Chem.*, **25**, 98 (1933).

used by bakers, may be added to promote fermentation. A blank test is run with 5 g. of yeast alone in 500 ml. of 4 per cent alcohol, the latter being used to prevent bacterial decomposition. At the end of the fermentation the original volume of each solution is restored by the addition of distilled water, and the solutions are clarified by filtration through Filter-Cel. Dry substance, protein, ash, and reducing sugars are determined in the fermented solutions, and corrections are applied for the values found in the blank. Total reducing sugars are expressed as glucose, as before.

The following data are thus obtained:

a: Dry substance — (ash plus protein) = total carbohydrates in 100 ml. of the solution before fermentation.

b: Dry substance — (ash plus protein) = carbohydrates in 100 ml. of the solution after fermentation (dextrin).

a — *b*: Total fermentable carbohydrates in 100 ml. solution (*F*).

c: Total reducing sugars, expressed as glucose, in 100 ml. of the solution before fermentation.

d: Total reducing sugars, expressed as glucose, in 100 ml. of the solution after fermentation.

c — *d*: Total fermentable reducing sugars, expressed as glucose, in 100 ml. of solution (*K*).

It remains now to calculate glucose (*D*) and maltose (*M*). This is done by means of the following formulas:

$$\begin{aligned} D \quad + \quad M &= F \\ D + 0.59\,M &= K \\ \hline 0.41\,M &= F - K \end{aligned}$$

$$M = \frac{F - K}{0.41}$$

$$D = F - M$$

The reducing ratio 0.59 for maltose was determined experimentally for the Soxhlet volumetric method.

Bryant and Jones have used this method for the analysis of commercial glucose, corn sugar, barley malt sirup, commercial dextrose, and commercial maltose. The results, expressed in percentage of total carbohydrates, are shown in Table CXXX. The specific rotations of the original products and of the unfermentable carbohydrates (dextrin), all on the dry basis, are also given (see p. 1002).

There is a large variation in the specific rotation of the dextrins, and some of the values are very much lower than the generally accepted values between 192 and 196, proving that the unfermented residue con-

tains varying quantities of maltodextrin and probably also of unfermentable disaccharides, as gentiobiose. The maltodextrins exhibit considerable reducing power, and for this reason the total reducing sugars in the corn sirups, determined as usually with Soxhlet's solution and expressed as glucose, do not check with the fermentable sugars, expressed the same way. This fact detracts greatly from the practical value of the method, although the specific rotation of the dextrins furnishes an approximate measure of the maltodextrin formed during the conversion.

TABLE CXXX

	Glucose	Maltose	Dextrin	$[\alpha]_D$ of product Dry Basis	$[\alpha]_D$ of Dextrin
	per cent	per cent	per cent		
Low conversion sirups	0–12.7	21.8–37.4	60.3–67.3	153–162	141–182
Medium conversion products	15.2–21.6	25.1–35.4	49.0–53.3	135–148	125–166
High conversion products	23.8–38.9	7.6–36.5	39.7–53.5	125–128	138–159
Corn sugar No. 70	76.0	9.7	14.3	54.5	.....
Commercial dextrose	94.5	1.6	3.9	54.7	.....
Commercial maltose	0.0	96.0	4.0	141	.....
Barley malt sirup	0.0	85.2	14.8	92	.....

Selective fermentation with specific yeasts has also been employed by a number of investigators. These methods require the greatest care in establishing the purity of the yeast strains, and it is always best to secure them from recognized institutions making a specialty of yeast cultures. Before use, they should be subcultured twice on malt agar for 48 hours each. The fermentations require as much as 2 weeks. For these reasons methods of this nature are of little use in routine work, but they may prove of distinct value in scientific investigation. One of them, designed particularly for the analysis of malt extracts but applicable also to other starch-conversion products, will be described as an example.

Method of McLachlan.[33] A solution containing 100 g. of the product in 1 liter is prepared. Fifty milliliters each of the solution is transferred to 8 fermentation tubes, which are sterilized on 3 successive days in a steam sterilizer, with rapid heating on the first day to destroy diastase. Two of the sterilized tubes are inoculated with *Saccharomyces exiguus*, 2 with Frohberg yeast, 2 with Saaz yeast, and 2 are used as a blank. The tubes are incubated at 26° C. for 14 days, being rotated on the fourth or fifth day. At the end of the fermentation pe-

[33] *Analyst*, 53, 583 (1928).

riod the contents of each tube are emptied, washed quantitatively into a 150-ml. beaker, and evaporated on the water bath to about 15 ml. After cooling, each solution is diluted to 50 ml. and filtered, and the specific gravity of the filtrate is determined. This is then converted into concentration by means of the appropriate solution factors. The difference between the concentration of the blank and the residue after fermentation with *S. exiguus* gives the glucose (and fructose, if present); that between *S. exiguus* and Frohberg yeast gives the maltose, and that between Frohberg yeast and Saaz yeast the other fermentable sugars. The residual solution after fermentation with Saaz yeast is polarized, and the dextrin calculated from the rotation and concentration, 180 being used as the specific rotation of the dextrin. Further information may be obtained by measuring the reducing power and polarization of all the solutions.

McLachlan found that malt extracts always contain fructose. Analyses of 12 malt extracts by the method described gave 10.1 to 25.5 per cent glucose plus fructose, 32.7 to 55.7 per cent maltose, 0.6 to 4.5 per cent other fermentable sugars, and 6.7 to 32.7 per cent dextrin.

Dextrin may also be determined directly by conversion into maltose and measurement of the decrease in rotation. This principle is employed in the following method.

Method of Fetzer, Evans, and Longenecker.[34] A specific rotation of 196 is assumed for the dextrin. One gram in 100 ml. would give an angular rotation of 3.92°, in a 2-dm. tube. Since 324 g. dextrin produces 342 g. anhydrous maltose, the angular rotation after the conversion will be reduced to 138.5 (specific rotation of maltose) × 1.0555 × 0.02, = 2.92°. Each drop of 1° in the rotation therefore indicates 1 g. dextrin in 100 ml. solution.

The malt solution for the conversion of the dextrin is prepared by transferring 187.5 g. of ground distiller's malt to a flask, and adding enough distilled water to cover the malt. The flask is placed in a water bath kept at about 16° C. for 1 hour. The infusion is filtered into a 250-ml. volumetric flask, and the filtrate returned until it runs clear. The malt on the filter is washed with small portions of cold, distilled water until nearly 250 ml. has been collected, and the volume is completed to the mark.

The angular rotation of the original corn sirup is determined in the usual manner, with a solution containing 25 g. sirup in 250 ml. solution.

For the determination of the dextrin, 25 g. of the sirup is weighed in a sugar weighing dish. Twenty milliliters of the malt solution is

[34] *Ind. Eng. Chem., Anal. Ed.*, 5, 81 (1933).

added, the dish is warmed to about 38° C., and the contents are thoroughly mixed and transferred to a flask of about 150-ml. capacity. The material remaining in the weighing dish is washed into the flask, first with a 20-ml. and then a 10-ml. portion of the malt solution, and finally with 10 ml. of water. A duplicate sample is prepared in the same way, and a third flask, with 50 ml. malt solution alone, is used as a blank. The three flasks are placed in a water bath heated to 63 to 64° C. Eight hours is usually sufficient to obtain a maximum decrease in the rotation, but it is advisable to establish the necessary time period for each new batch of malt used. Tests with purified dextrin have shown that, when the maximum decrease in rotation is reached, the conversion is more than 98 per cent complete.

At the end of the conversion the flasks are placed for at least half an hour on a steam bath to coagulate the protein. The solutions are then cooled, and the protein is filtered off and washed. The filtrates from the two samples are diluted to 250 ml., and the malt blank to 100 ml. The angular rotation of the solutions is determined, and two-fifths of the rotation of the malt blank is deducted from the average rotation of the two samples. The grams of dextrin in 10 g. of the corn sirup are numerically equal to the difference between the angular rotation of the original corn sirup solution and the corrected angular rotation found after the conversion.

To find the maltose and glucose in the corn sirup, the total solids in the original sirup are determined by the toluene distillation method described on p. 42, and the ash and protein are deducted. This gives the total carbohydrate solids. If the maltose is designated by y, the glucose by z, the total carbohydrate solids by T, the dextrin previously found by D, and the angular rotation of the original sirup by P, then y and z may be calculated by means of the following equations:

$$y + z = T - D$$

$$2.770\,y + 1.054\,z = P - 3.92\,D$$

whence

$$z = \frac{2.77\,T + 1.15\,D - P}{1.716}$$

and

$$y = T - D - z$$

In a 2-dm. tube, 2.770 is the angular rotation of 1 g. maltose and 1.054 that of 1 g. glucose in 100 ml. solution.

Analyses of three corn sirups by this method gave the following results for glucose, maltose, and dextrin, expressed as percentage on carbohydrate solids:

Sirup	Glucose	Maltose	Dextrin	Specific Rotation of Sirup
Low conversion sirup.....	11.23	38.32	50.45	157.9
Medium conversion sirup..	18.26	46.01	35.73	143.4
High conversion sirup....	29.16	43.26	27.58	129.3

The maltose figures are higher, and the glucose figures lower, than those of Bryant and Jones (p. 1000) for the same types of sirup, as would be expected from the fact that in the latter method the maltodextrin is classified as dextrin, while in the present method it is considered to consist of maltose and of dextrin of specific rotation equal to 196.

In this method, the sum of the glucose and maltose, expressed as glucose, checks closely with the total reducing sugars, determined by the usual method with Soxhlet's solution, and also expressed as glucose, while in the method of Bryant and Jones there are often wide discrepancies between the two. Nevertheless, since the degradation of starch by acids is known to be a gradual one, the classification of the products intermediate between dextrin and maltose is merely a conventional matter.

The same observation applies also to the method to be next described, in which glucose, maltose, and dextrin are each determined directly by reduction methods.

Method of Steinhoff.[35] The selective estimation of glucose, in the presence of reducing disaccharides, with a modified Barfoed solution, as developed by Steinhoff, has been referred to on p. 820. In its application to starch-conversion products, the sum of maltose and glucose is determined with Soxhlet's solution and corrected for the glucose previously found. The total carbohydrates are determined by copper reduction after acid hydrolysis.

The details of the method are as follows. A convenient quantity of the corn sirup, containing about 10 g. of solids, is weighed out and diluted to 1 liter.

(*a*) For the selective determination of the glucose, 10 ml. of the solution is transferred to a 200-ml. Erlenmeyer flask. Ten milliliters of the blue Soxhlet's solution A, 20 ml. of a solution containing 500 g. sodium acetate per liter, and 10 ml. of water are added, making 50 ml. solution in all.

(*b*) For the estimation of glucose plus maltose, another 10-ml.

[35] *Z. Spiritusind.*, 56, 64 (1933). See also Sichert and Bleyer, *Z. anal. Chem.*, 107, 328 (1936).

portion of the original solution is mixed in a similar Erlenmeyer flask with 10 ml. each of Soxhlet's solutions A and B and 20 ml. of water.

(*c*) To convert the total carbohydrates into glucose, 50 ml. of the original solution and 25 ml. of 3 *N* hydrochloric acid are measured into a 100-ml. volumetric flask and heated for 2½ hours in a boiling-water bath. The flask is cooled, the free acid neutralized with sodium hydroxide, phenolphthalein being used as indicator, and the volume is completed to the mark. Twenty milliliters of this solution is mixed in a third Erlenmeyer flask with 10 ml. each of Soxhlet's solutions A and B, and 10 ml. of water.

All three flasks are placed in a boiling-water bath for exactly 20 minutes. Without filtration, the cuprous oxide is dissolved by the addition of 10 ml. of 10 per cent hydrochloric acid to the still hot solution, and the excess acid is neutralized with 10 ml. of 8 per cent sodium bicarbonate solution. An excess of *N*/10 iodine solution is run in at once, and the flask is gently rotated until the white precipitate formed at first is completely dissolved and the solution appears dark green. After cooling, the excess iodine is titrated with *N*/10 sodium thiosulfate, starch solution being used as indicator. The thiosulfate required should be at least 5 ml. A blank titration is unnecessary.

The milligrams of glucose, maltose hydrate, and dextrin, corresponding to the iodine required for oxidation of the cuprous oxide, are found from Table CXXXI.

Column 2 gives the milligrams of glucose corresponding to the milliliters of *N*/10 iodine solution used. The milligrams of maltose are calculated by first finding from column 3 the milliliters of *N*/10 iodine solution which would have been used if Soxhlet's solution had been used instead of the copper acetate reagent in experiment (*a*), deducting these from the milliliters of iodine solution required in experiment (*b*), and finding the corresponding milligrams of maltose from column 4. Column 5 gives the milligrams of total glucose corresponding to the milliliters of *N*/10 iodine solution used in experiment (*c*). Since 9 parts dextrin give 10 parts glucose upon hydrolysis, the sum of the milligrams of glucose and maltose found in experiments (*a*) and (*b*) is deducted from the milligrams of glucose found in experiment (*c*), and the difference is multiplied by 0.9 to find the milligrams of dextrin.

Example. In experiment (*a*) 3 ml. of *N*/10 iodine solution was required for the oxidation of the reduced copper. This corresponds to 11.2 mg. glucose. Experiment (*b*) required 8 ml. iodine solution. From this must be deducted 3.6 ml., corresponding to the reducing effect of the glucose. This leaves 4.4 ml., corresponding to 23.7 mg. maltose. Experiment (*c*) required 22 ml. of iodine solution, equal to 74.5 mg. total glucose. Deducting from this

TABLE CXXXI

1	2	3	4	5
ml. *N*/10 Iodine	mg. Glucose, by Copper Acetate Reagent	ml. *N*/10 Iodine to Be Deducted	mg. Maltose hydrate, from Difference	mg. Total Glucose
1	6.1	1.9	5.0	3.2
2	8.6	2.7	10.5	6.3
3	11.2	3.6	16.0	9.4
4	13.4	4.3	21.5	12.6
5	15.6	4.9	27.0	15.9
6	18.1	5.7	32.5	19.2
7	20.5	6.4	38.0	22.4
8	23.2	7.3	43.5	25.6
9	25.8	8.1	49.0	28.9
10	28.4	8.9	55.0	32.3
11	31.2	9.7	60.5	35.7
12	34.2	10.6	66.0	39.0
13	37.3	11.5	72.0	42.4
14	40.5	12.4	78.0	45.8
15	46.8	14.3	83.5	49.3
16	52.8	16.0	89.0	52.8
17	58.6	17.7	95.0	56.3
18	66.0	19.8	101.0	59.8
19	73.4	21.8	107.0	63.3
20	80.0	23.4	112.5	66.9
21	88.4	25.4	118.5	70.7
22	96.2	27.3	124.5	74.5
23	....	....	130.5	78.5
24	....	....	136.5	82.6
25	....	....	142.5	86.6
26	....	....	148.5	90.7
27	....	....	154.5	94.8

11.2 mg. glucose and 23.7 mg. maltose, and multiplying by 0.9, we obtain 35.6 mg. dextrin. The percentage composition of the total carbohydrates is therefore 15.9 per cent glucose, 33.6 per cent maltose, and 50.5 per cent dextrin.

Using the commonly accepted values for the specific rotation of glucose, maltose, and dextrin, Steinhoff found that the specific rotation calculated from the percentages of the constituents checked within —1.1 to +2.7 per cent with the specific rotation of the sirups analyzed.

In a critical study of Steinhoff's method, Zerban and Sattler[36] found that maltose has a slight reducing effect on the copper acetate reagent, which must be corrected for; that the iodine titration procedure of Steinhoff gives erratic results because the iodine is partly volatilized when added to the hot solution, and that there are other objections to the method. When proper corrections are applied (see p. 1014) the method will give more accurate results.

[36] *Ind. Eng. Chem., Anal. Ed.*, 10, 669 (1938).

Application to Other Sugar Mixtures. The general principles of combining measurements of rotation, reducing power, and direct determination of one or more constituents in analyzing mixtures of three sugars has been sufficiently indicated, and additional examples need not be given. Such schemes of analysis obviously admit of unlimited extension. If one of the three sugars is a pentose or methylpentose, its percentage may be determined from the yield of furfural- or methylfurfural phloroglucide; mannose may be determined from the yield of phenylhydrazone; lactose or galactose from the yield of mucic acid; raffinose by the method of inversion; etc. In combining the results of such direct determinations with those of polarization and reducing power, the chemist must consider in each case the limitations of the methods used and the extent to which experimental errors are multiplied in the calculation.

The final test of accuracy consists in applying the method to the analysis of mixtures containing known amounts of the several sugars, and this verification should be made whenever possible.

III. Analysis of Mixtures Containing More Than Three Sugars

Schemes of analysis have also been proposed for the analysis of mixtures containing four or more sugars, in which case, however, at least two must usually be determined by direct means.

As an illustration of a method for analyzing a mixture of four sugars, containing g per cent of glucose, f per cent fructose, s per cent sucrose, and x per cent xylose, the following outline is presented.

$$0.793\,g - 1.397\,f + s + 0.283\,x = P\begin{pmatrix}\text{polarization of a sucrose normal}\\ \text{weight upon a saccharimeter}\end{pmatrix} \quad (1)$$

$$g + 0.915\,f + 0.983\,x = R \text{ (total reducing sugars as glucose)} \quad (2)$$

$$s = S \text{ (sucrose determined by method of inversion)} \quad (3)$$

$$x = X \text{ (xylose determined from yield of furfural phloroglucide)} \quad (4)$$

Substituting the known values of S and X in (1) and (2) gives:

$$0.793\,g - 1.397\,f = P - S - 0.283\,X \quad (5)$$

$$g + 0.915\,f = R - 0.983\,X \quad (6)$$

Multiplying (6) by 0.793 and combining with (5) gives:

$$f = \text{per cent fructose} = \frac{S + 0.793\,R - P - 0.498\,X}{2.123}$$

$$g = \text{per cent glucose} = R - 0.915\,f - 0.983\,X$$

The application of such formulas as the above to the analysis of complicated mixtures of sugars, however, usually involves such a combination and multiplication of experimental errors that a scheme of calculation perfectly correct in theory is shown in practice to be almost valueless.

A few other examples of methods for the analysis of products containing four or more carbohydrates will be given.

Method of Jackson, Mathews, and Chase for the Analysis of Natural Honeys and Fruit Juices.[37] If sucrose is estimated in these products by the Clerget method with the use of invertase, and glucose and fructose by a combination of the Lane and Eynon and the modified Nijns method (p. 975), as well as by a combination of the Lane and Eynon method with direct polarization, corrected for that due to sucrose (p. 983), the latter method usually gives either a higher or lower ratio between fructose and total reducing sugars than the former. This shows that at least one other optically active or reducing substance is present besides sucrose, glucose, and fructose. If it is assumed that glucose and fructose are the only reducing substances, the difference between the ratio of fructose and reducing sugars, as determined by the two methods, presents an empirical measure of the additional optically active substance or substances. Honeys are known to contain dextrins, and by assigning an arbitrary specific rotation to them, their quantity can be calculated by means of the following formula:

$$\text{Per cent optically active substance (dextrin)} = \frac{VcD\Delta R}{100\,gT}$$

where V is the volume of solution, containing g grams of substance, used for the direct polarization; c the saccharimetric normal weight of the optically active substance, based on its specific rotation and that of sucrose; D the number of volumes to which 1 volume of the polarized solution has been diluted for the Lane and Eynon titration; T the Lane and Eynon titer; and ΔR the difference between the ratio of fructose to total reducing sugar by the combined reduction method and that by the combined reduction and polarization.

If ΔR is positive, the optically active substance is dextrorotatory; and if it is negative, the presence of a levorotatory constituent is indicated.

Table CXXXII gives the analysis of a number of honeys made by this method. The specific rotation of the honey dextrin is assumed to be +150, and c in the above formula therefore equals 11.5.

[37] *Bur. Standards J. Research*, 9, 597 (1932).

TABLE CXXXII

Analyses of Honeys

Principal Floral Source of Honey	Dry Substance	Sucrose	Reducing Sugars	Fructose	Ratio: Fructose / Reducing Sugar	Mathews Ratio: Fructose / Reducing Sugar	Apparent Dextrins
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Sumac	82.54	3.26	72.13	37.53	51.9	43.2	5.81
White clover	82.42	1.69	76.09	39.26	51.6	46.9	3.32
Buckwheat	81.08	.44	75.53	38.97	51.6	46.3	3.71
Blend No. 1	82.42	1.61	78.69	42.02	53.4	50.3	2.26
Alfalfa	85.68	4.73	76.05	39.63	52.1	44.8	5.15
Sourwood	82.46	1.41	69.17	38.94	56.3	44.8	7.99
Fireweed	84.48	4.54	74.76	40.97	54.8	47.4	5.12
Gallberry	83.24	.83	73.20	41.14	56.2	48.5	5.21
Goldenrod	82.16	.68	77.08	39.93	51.8	47.0	3.43
Blend No. 2	84.84	1.65	77.81	39.92	51.3	45.8	3.97
Honey dew (incense cedar)	86.44	2.90	61.05	28.95	47.4	11.4	20.43
Melezitose honey	83.50	.30	57.61	32.57	56.5	27.0	15.70

In all these samples, except the melezitose honey, the sum of sucrose, glucose, fructose, and apparent dextrin approaches the total dry substance within about 2 per cent. In the melezitose honey, however, there is a shortage of about 10 per cent, due to the presence of melezitose. By assuming that the total carbohydrates in this honey constitute the same percentage of the dry substance as in the other honeys, the melezitose can be estimated approximately. Assigning a specific rotation of +150 to the honey dextrin as before, and of +87.8 to the melezitose, the quantity of both can be calculated by two equations, one based on ΔR and the other on the difference between the total carbohydrates and the sum of sucrose, glucose, and fructose. These equations are perfectly analogous to those previously shown in this chapter. The solution of these equations gives 3.9 per cent dextrin and 20.2 per cent melezitose. This honey represents a good example of the analysis of a mixture of five carbohydrates, with a fair degree of approximation. In the interpretation of such analyses it must always be kept in mind that they are based on assumptions of restricted validity.

In fruit juices, analyses of which are shown in Table CXXXIII, the difference between the ratios of fructose to reducing sugars by the two methods employed is small, except for peach juice and cranberry juice. In these, as well as in apple juice, the difference is negative, indicating the presence of a levorotatory substance. As long as the nature of these substances is not known, the analyses cannot be definitely interpreted. Nevertheless, the ΔR constitutes a valuable criterion in the examination of these products.

TABLE CXXXIII

Analyses of Fruit Juices

Fruit Juice	Dry Substance by Refractometer	Sucrose	Reducing Sugars	Fructose	Ratio: Fructose / Reducing Sugar	Mathewe Ratio: Fructose / Reducing Sugar	ΔR	Total Sugar Purity
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Peach, "Old Gold"	16.50	8.62	3.17	1.82	57.4	63.8	— 6.4	71.5
Apple, "Delicious"	13.14	1.80	9.63	6.58	68.3	69.8	— 1.5	87.0
Pear, "D'Anjou"	17.06	1.24	9.99	8.88	88.9	86.7	2.2	65.8
Lemon	8.33	.40	2.03	1.07	52.9	51.8	1.1	29.2
Orange	12.07	4.50	5.07	2.65	52.2	51.7	.5	79.3
Grapefruit	13.84	4.03	6.05	3.17	52.5	51.3	1.2	72.8
Grapes, "Almeria"	18.05	.19	16.28	8.32	51.1	48.6	2.5	91.2
Strawberry	7.63	.82	4.09	2.20	53.8	51.2	2.6	66.4
Cranberry	9.93	.24	5.18	.82	15.8	30.5	−14.7	54.6
Tomato	4.67	0	2.79	1.58	57.7	56.3	1.4	59.7

Method of Nanji and Beazeley for Analyzing Mixtures of Starch-Conversion and Cane-Sugar Products.[38] The principal carbohydrates present in such mixtures are five in number: sucrose, glucose, fructose, maltose, and dextrin. Nanji and Beazeley proceed as follows to estimate each one of them. The total reducing power, due to glucose, fructose, maltose, and dextrin, is determined by Fehling's solution, and the result expressed as glucose. The total aldoses are determined by oxidation with iodine in alkaline solution (pp. 895–901), and are also expressed as glucose. A correction is applied for the partial oxidation of the fructose under the experimental conditions used. The difference between the total reducing sugars and the aldoses gives a measure of the fructose. For the estimation of the sucrose, Nanji and Beazeley boil for 10 minutes in the presence of 10 g. citric acid in 100 ml. solution (to avoid the hydrolysis of maltose and dextrin), measure the reducing power after inversion, and calculate the sucrose as usual from the additional invert sugar formed. It is always preferable, however, to employ inversion with invertase rather than with acid. The dextrin is found by fermenting a 10 per cent solution of the product with Frohberg yeast, determining the specific gravity of the dealcoholized solution after completion to the original volume, and calculating the concentration of dextrin by means of the solution factor 4.00, after correcting for the ash and yeast. The sum of the glucose and maltose is estimated by (1) measuring the rotation of the original and of the fermented 10 per cent solution and (2) by the difference between the total carbohydrates, based on the density of the original solution after correcting for ash and protein, and the sum of sucrose, fructose, and dextrin. Two simulta-

[38] *J. Soc. Chem. Ind.*, **45**, 220T (1926).

neous equations are then set up, from which the glucose and the maltose may be calculated.

There are so many sources of error, previously referred to, in this method, as in others of this type, that the results can be considered only as a rough approximation. This applies also to the method to be described next.

Kolthoff-Kruisheer Method. A somewhat simpler method than the one just described has been proposed by Kolthoff[39] and elaborated by Kruisheer.[40] It is assumed that the only carbohydrates in the cane-sugar product are sucrose and invert sugar. The dextrin in the starch product is considered to be non-reducing, while the maltose and other reducing sugars are calculated as glucose. The method rests on the determination of total reducing sugars (R) and of fructose (F) by the modified Luff-Schoorl procedure, which has the advantage that with the copper reagent used there is no difference in the reducing power of glucose and fructose. The Kolthoff-Kruisheer method (p. 902) is used to oxidize the aldoses with hypoiodite, leaving fructose as the only reducing substance. R and F are measured (a) in the original product (R_1 and F_1); (b) after heating 50 ml. of the original solution with 5 ml. of 30 per cent hydrochloric acid in a 100-ml. flask to 68–70° C. for 10 minutes, cooling, neutralizing, cooling again, and making up to volume (R_2 and F_2); (c) after heating 50 ml. of the original solution with 5 ml. of 30 per cent hydrochloric acid and 25 ml. of water for 1 hour in a briskly boiling water bath, cooling, neutralizing, cooling again, and making up to 100 ml. (R_3 and F_3). It is claimed that the weak inversion (b) hydrolyzes only the sucrose and has a negligible effect on the maltose and dextrin. The strong inversion (c) converts maltose and dextrin into glucose. The composition of the mixture before and after the treatments with acid is illustrated by the following scheme:

Cane-Sugar Product	Starch-Conversion Product
Original Solution	
Sucrose + Glucose + Fructose (F_1)	Glucose + Maltose + Dextrin
After Weak Inversion	
Glucose (G_A) + Fructose (F_2)	Glucose + Maltose + Dextrin
After Strong Inversion	
Glucose (G_B) + Fructose (F_3)	Glucose (G_Z)

From the values of R_1, F_1, R_2, F_2, R_3, and F_3 the following data may be calculated: The sucrose originally present is 0.95 ($R_2 - R_1$), or 1.9

[39] *Z. Untersuch. Nahr. u. Genussm.*, **45**, 131 (1923).
[40] *Z. Untersuch. Lebensm.*, **58**, 261 (1929).

($F_2 - F_1$). As previously noted, it is always better to determine sucrose by inversion with invertase rather than with acid. The invert sugar in the original product equals $2F_1$.

After the strong inversion according to procedure (*c*),

$$R_3 = G_B + F_3 + G_Z, \quad \text{or} \quad G_Z = R_3 - G_B - F_3$$

If, as postulated, only sucrose and invert sugar are present in the cane-product portion of the mixture, $G_B = G_A = F_2$. Therefore,

$$G_Z = R_3 - F_2 - F_3$$

Theoretically, the total quantity of carbohydrate solids in the starch-product portion should be smaller than G_Z, since both maltose and dextrin take up water during the conversion to dextrose. Kruisheer found, however, by the analysis of starch-conversion products, that G_Z is practically equal to the total carbohydrate solids based on the specific gravity of the product. This is evidently due to incomplete hydrolysis of the dextrin, or to the formation of reversion products. Hence

$$\text{Total starch product solids } (Z) = R_3 - F_2 - F_3$$

The dextrin equals the total glucose after strong hydrolysis, less the glucose after weak hydrolysis:

$$\text{Dextrin } (D) = (R_3 - F_3) - (R_2 - F_2)$$

The glucose equivalent to the glucose plus maltose attributable to the starch-conversion product may be calculated by deducting from the total carbohydrate solids the sum of sucrose, invert sugar, and dextrin.

Great caution must be exercised in interpreting the results obtained, and the various assumptions made must always be kept in mind. Reicher[41] obtained satisfactory results by the above method in the analysis of mixtures of sucrose, invert sugar, and commercial glucose.

Shapiro's Method for Mixtures of Sucrose, Glucose, Fructose, and Maltose.[42] In this method the fructose is determined by copper reduction after oxidation of the aldoses with hypoiodite (method of Kolthoff-Kruisheer (p. 902), the maltose by the procedure of Shapiro and Proferansova (p. 900), the sucrose by Shapiro's method (p. 855), the total reducing sugars by the method of Luff-Schoorl (p. 832), and the glucose by the difference between the total reducing sugars and the sum of fructose and maltose.

[41] *Gaz. cukrownicza*, 77, 5 (1935).
[42] *Z. Ver. deut. Zucker-Ind.*, 86, 6 (1936).

Method of Zerban and Sattler for Mixtures of Glucose, Fructose, Maltose, and Lactose.[43] The sum of glucose and fructose is found by Steinhoff's copper acetate reagent (p. 820), the reduced copper being estimated according to Shaffer and Hartmann (p. 836). The apparent fructose is determined by the procedure of Jackson and Mathews (p. 824). A correction is applied for the reducing effect of maltose and lactose on both the Steinhoff and the Jackson and Mathews reagent, and then the glucose and fructose are calculated from the two equations obtained. The lactose is determined by copper reduction after the other sugars have been removed by fermentation with baker's yeast. The total reducing sugars are determined with Steinhoff's copper tartrate method (p. 1005). The maltose is found by the difference between the total reducing sugars and the sum of glucose, fructose, and lactose. A series of approximations is necessary to get the final results. For the details of these calculations the chemist is referred to the original paper. If sucrose is also present, in addition to the other sugars, it is determined by copper reduction before and after inversion with invertase. This method has been applied to the determination of the sugars present in baked bread.[44]

It is scarcely necessary to remark that, in working with unknown mixtures of sugars, each of the constituents present must by identified by careful qualitative tests before beginning the analysis.

ANALYSIS OF SUGAR MIXTURES BY FRACTIONAL DISTILLATION OF THE METHYLATED SUGARS

A method for analyzing sugar mixtures which is based on an entirely different principle from those previously described has been devised by Hurd and Cantor.[45] Its primary purpose is to separate different classes of sugars from one another, such as monosaccharides from disaccharides, and either one of these from trisaccharides, or pentoses from hexoses, etc. The sugar mixture is methylated by an indirect procedure. It is first fully acetylated, then the acetyl attached to the carbonyl group is successively replaced by chlorine and by methoxyl; the remaining acetyl groups are split off and the resulting product is methylated. The mixture of methylated sugars is subjected to fractional distillation in a high vacuum (0.001 to 0.008 mm. pressure). Under these conditions the monosaccharides contained in hydrol, the mother liquor left after the crystallization of glucose from hydrolyzed corn starch, distilled over

[43] *Ind. Eng. Chem., Anal. Ed.*, **10**, 669 (1938).
[44] Rice, *Cereal Chem.*, **15**, 672 (1938).
[45] *J. Am. Chem. Soc.*, **60**, 2677 (1938).

at a temperature up to 110° C., the disaccharides at 160 to 190° C., and the residue left contained higher saccharides, probably trisaccharides. In a mixture of xylose and glucose the former distilled over at a temperature below 75° C. The various fractions obtained can be used for identifying the components of each. The method is tedious, requires careful technique, and, in its present form, is not adapted for routine analyses.

For a description of other methods and schemes which have been proposed for analyzing different mixtures of sugars, the chemist is referred to the special literature on this subject.[46]

[46] See Lippmann, "Chemie der Zuckerarten," 3rd ed., Vol. I, pp. 616–623, 894–899; Van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydsäuren," 1920; Bruhns, "Zuckerbestimmungen in allerlei Stoffen"; *Deut. Zuckerind.*, 57, 971, 1008; 58, 287, 349, 513, 548, 921, 939; 59, 483, 593 (1932–34).

CHAPTER XVII

SELECTED METHODS FOR MISCELLANEOUS CARBOHYDRATE PRODUCTS

The present chapter will give some practical applications of the principles and methods previously described, and other selected procedures used in technical sugar analysis. A large number of such applications have already been considered, and these will be passed over. The methods will be grouped under three main divisions of products: (1) sugar-factory products; (2) starch products; (3) miscellaneous food products.

SUGAR-FACTORY PRODUCTS

In the first part of this section definitions of the more important terms used in sugar technology and descriptions of illustrative commercial methods will be given. For methods employed exclusively in sugar-factory control the books of Spencer-Meade, of the Association of Hawaiian Sugar Technologists, of the Java Sugar Experiment Station, of Frühling-Spengler, Deerr, Saillard, Sidersky, Wohryzek, and others should be consulted.

The nature and quantity of individual non-sugars or groups of non-sugars in commercial sugars, sirups, etc., frequently exert an important influence on their quality and suitability for commercial use, and appropriate methods for the determination of these non-sugars are taken up in the second part of this section.

The third part of the section deals with miscellaneous physical, chemical, and bacteriological tests used for the evaluation of refined and other direct consumption sugars for technical and commercial purposes.

I. General Terms and Methods

Pol. Although cane products, and to a lesser extent beet products, contain other optically active substances besides sucrose, the technical accounting of sugar factories in many countries is still based on direct polarization, rather than on actual sucrose content. In some countries, especially in Cuba, the figure obtained is nevertheless designated as "sucrose." In order to end the ensuing uncertainty, the International

Society of Sugar Cane Technologists has adopted the term "pol," defined as follows: "The value determined by single or direct polarization of the normal weight in a saccharimeter. The term is used in calculations as if it were a real substance."

Purity. The purity, also called "quotient of purity," "quotient," "degree of purity," or "exponent," of a juice, sirup, molasses, sugar, etc., is the percentage of saccharine matter in the total solid matter of the product. The term has been variously interpreted, and the chemist must distinguish carefully between the different concepts, defined as follows by the International Society of Sugar Cane Technologists:

The *true purity* is the percentage proportion of *sucrose* in the *dry substance* (solids determined by drying).

The *gravity purity* is the percentage proportion of *sucrose* in the *gravity solids*, which are numerically equal to the degree Brix.

The *apparent purity*, or "purity" without further qualification, is the percentage proportion of *pol* in the *gravity solids*, which are numerically equal to the degree Brix.

If the refractometer is used for determining the apparent total solids, these are characterized as refractometer Brix (R. Br.), or refractometer solids, and purities based on them must be specially designated, to avoid confusion.

Example. A sugar-cane molasses gave upon analysis the following results:

Dry substance (total solids by drying)	75.10 per cent
Gravity solids, by degrees Brix	77.10 per cent
Total solids by refractometer	74.20 per cent
Pol (direct polarization)	42.20 per cent
Sucrose (by method of inversion)	45.70 per cent

$$\text{True purity} = \frac{100 \times 45.70}{75.10} = 60.85$$

$$\text{Gravity purity} = \frac{100 \times 45.70}{77.10} = 59.27$$

$$\text{Apparent purity} = \frac{100 \times 42.20}{77.10} = 54.73$$

$$\text{Purity, Sucrose/R. Br.} = \frac{100 \times 45.70}{74.20} = 61.59$$

$$\text{Purity, Pol/R. Br.} = \frac{100 \times 42.20}{74.20} = 56.87$$

In the United States beet sugar industry the refractometer solids are considered to be equal to the dry substance for purposes of routine fac-

tory control, and the percentage proportion of sucrose in the refractometer solids is designated as true purity.

Purity calculations are much simplified by such mechanical aids as the nomon of Blake,[1] the slide rule of Bastone,[2] or Horne's purity table mounted on rollers.[3]

Purity Determination in Beet-House Sirups by Conductivity Measurements. This method for rapid routine determinations of the true purity, developed by Zisch,[4] utilizes the depressing effect of non-electrolytes on the conductance of a strong acid. First the total sugars (sucrose and raffinose) are determined in a number of representative samples by the inversion method, and also the specific conductance at 25° C. of a dilute hydrochloric acid solution, with and without the addition of a solution of the sugar product, by the method described below. The difference between the two conductance determinations gives the "conductivity depression." This is plotted against the total sugars expressed in percentage of the refractometer solids (total sugar purity) of the samples. As long as the ratio between ash and total sugars in the samples is practically constant, a straight-line relationship is found, which is used subsequently to obtain the total sugar purity of unknown samples from the conductivity depression.

A sample of sirup, containing 5 g. dry substance by refractometer, is transferred to a 100-ml. flask, diluted to 50–60 ml. with conductivity water, exactly 25 ml. of dilute hydrochloric acid (25 ml. of concentrated acid diluted to 1 liter) is added, and the volume is completed to 100 ml. with conductivity water. In another flask, 25 ml. of the dilute acid is made up directly to 100 ml. with conductivity water. The specific conductance of the two solutions is determined (see p. 548) at 25° C., and the total sugar purity corresponding to the conductivity depression is found from the graph.

The apparent purity (percentage ratio of pol to refractometer solids) of the sample is also determined, and the true purity (x) as well as the percentage of raffinose in the dry substance by refractometer (y) is then calculated by means of the following equations, in which P denotes the apparent purity and T the total sugar purity:

$$\begin{aligned} x + 1.85\,y &= P \\ x + \quad y &= T \\ \hline 0.85\,y &= P - T \\ y &= \frac{P - T}{0.85} \\ x &= T - y \end{aligned}$$

[1] *Intern. Sugar J.*, **20**, 73 (1918).
[2] *Ind. Eng. Chem.*, **16**, 176 (1924).
[3] *Ind. Eng. Chem.*, **14**, 944 (1922).
[4] *Facts About Sugar*, **25**, 741 (1930); **26**, 299 (1931).

If the ash content of the products exceeds 10 per cent, all the conductance determinations should be made with 50 instead of 25 ml. of hydrochloric acid.

This method makes it possible to determine the true purity of a beet sirup in a very short time with sufficient accuracy for factory control, whereas the chemical method requires nearly 2 hours.

The purity of sugar-cane or sugar-beet juices is often loosely applied to the entire cane or beet.

Numerous tables and formulas have been calculated for converting apparent into true purities, but these can be used only upon the special classes of products and for the particular locality for which they have been designed. The same is true also of the conversion of gravity solids or refractometer solids into dry substance.

Determination of Ash. The determination of ash is of great importance in the technical analysis of sugar products. Several methods of the Association of Official Agricultural Chemists are given.[5]

Carbonated Ash, Method I. Heat 5–10 g. of the sample in a 50 to 100-ml. platinum dish at 100° C. until the water is expelled, add a few drops of pure olive oil, and heat slowly over a flame until swelling ceases. Place the dish in a muffle and heat at low redness until a white ash is obtained. Treat the residue with a little ammonium carbonate solution, re-evaporate, and heat again in the muffle at a very dull red heat to constant weight.

Carbonated Ash, Method II. Carbonize 5–10 g. of the sample in a 50 to 100-ml. platinum dish at about 525° C. and treat the charred mass with hot water to dissolve the soluble salts. (In the case of low-purity products the addition of a few drops of pure olive oil, as in Method I, may be desirable.) Filter through an ashless filter, ignite filter and residue to a white ash; add the filtrate of soluble salts, evaporate to dryness, and ignite gently. Treat the residue with a little ammonium carbonate solution, re-evaporate, and heat again in the muffle at a very dull red heat to constant weight.

According to Wichmann[6] more concordant results are obtained if, after the addition of the ammonium carbonate and evaporation to dryness, the mass is heated in an electric muffle oven to 260° C. At this temperature the magnesium carbonate is not decomposed.

When it is desired to make a quantitative analysis of the ash, Method II is preferable, because it facilitates complete combustion of the carbon formed during the incineration.

Soluble and Insoluble Ash. Add water to the carbonated ash in the platinum dish, heat nearly to boiling, filter through an ashless filter, and wash with hot water until the combined filtrate and washings measure about 60 ml. Return

[5] "Methods of Analysis, A.O.A.C.," 4th ed., p. 465, 1935. The treatment with ammonium carbonate was dropped in 1940.

[6] Private communication.

the filter and contents to the platinum dish, ignite carefully, cool, and weigh. Calculate the percentages of water-soluble and water-insoluble ash.

It has long been known that during the incineration of sugar products a part of the mineral matter is lost by volatilization. This is true particularly of ammonium salts, nitrates, chlorides, and sulfates. On the other hand, organic acids are converted into carbonates. For this reason the carbonated ash is not an exact measure either of the total mineral salts or of the total inorganic and organic salts present originally in the product, but is merely a conventional figure.

Other methods have been proposed from time to time, which seek to avoid or minimize the errors due to the volatilization of chlorides and sulfates. Browne and Gamble[7] have shown that the addition of alkali carbonates decreases this loss, but does not entirely prevent it. Magnesium oxide, zinc oxide, oxalic acid, and benzoic acid have been recommended to facilitate the ashing operations and to produce more uniform results, but none of these methods has come into general use.

Scheibler[8] introduced incineration with a small quantity of sulfuric acid. In this method the fixed bases are recovered as sulfates, together with phosphates and silicates, while the organic matter is completely volatilized.

Sulfated Ash Method.[9] Weigh 5 g. of the sample into a 50 to 100-ml. platinum dish, add 5 ml. of 10 per cent sulfuric acid, ignite until the sample is well carbonized, and then burn in a muffle at about 550° C. Cool, add 2–3 ml. of 10 per cent sulfuric acid, evaporate on a steam bath, dry on a hot plate, and again ignite at 550° C. to constant weight. Express the result as percentage of sulfated ash.

Valdez and Camps-Campins[10] recommend heating of the carbonized and resulfated mass for 1 hour in a muffle at 1480° F. (804° C.). At this temperature the salts of fixed bases are converted into sulfates except iron and aluminum salts which are obtained as oxides of these metals. No account is taken of phosphates, which appear to be converted into sulfates and pyrophosphates.

The same authors have also shown that large quantities of sugar of very low ash content, as refined sugars, can be easily carbonized by preparing a solution of about 50 Brix, adding a few milliliters of sulfuric acid, and dropping this mixture slowly, from a pipette with a piece of rubber tubing and a screw clamp on top, into a platinum dish

[7] *Facts About Sugar*, 17, 552 (1923).
[8] *Z. Rübenzuckerind.*, 14, 188 (1864).
[9] " Methods of Analysis, A.O.A.C.," 5th ed., p. 487, 1940.
[10] *Ind. Eng. Chem., Anal. Ed.*, 9, 35 (1937).

heated over a small, open flame. As much as 100 g. of sugar can be carbonized in this manner in a 100-ml. dish.

Instead of the expensive platinum, silica or even porcelain dishes may be used for the determination of sulfated ash, and dishes of nickel or resistant steel for carbonated ash.

Scheibler[11] found that in the case of beet products, 10 per cent must be deducted from the weight of sulfated ash to convert it into the equivalent weight of carbonated ash. This correction factor has been adopted for commercial analyses by the International Commission for Uniform Methods of Sugar Analysis, and is in official use in most countries.

It has been shown, however, that the correction factor actually varies within wide limits, for both beet and cane products. Schlegel and Weber[12] have reported values from 9.39 to 20.24 per cent for centrifugal cane raw sugars, and from 6.98 to 20.65 per cent for cane molasses, the average being about 14 per cent. In some cases correction factors as high as 25 per cent have been found. It is evident, therefore, that there is no simple relation between sulfated and carbonated ash, and that the use of any average figure may lead to large errors. For this reason the Association of Official Agricultural Chemists, and also the Java Sugar Experiment Station report sulfated ash without any correction.

Ash Determination by Electrical Conductivity Measurements. Chemical ash determinations require a great deal of care and considerable time and fuel, so that large numbers of routine tests are out of the question in the average factory laboratory. As a quick, alternate method, Reichert proposed in 1889 to use the electrical conductivity as an indirect measure of the ash content. The idea was taken up by others from time to time, but it was not until 1925, when a simple apparatus was designed by Tödt,[13] that the conductivity method became of practical importance.

The conductivity indicates the concentration of ionized salts present in an impure sugar solution. Although this is something quite different from the ash as defined above, it has been possible to correlate the two, and to establish equations by which the ash may be calculated from the conductivity, with sufficient accuracy for practical purposes.

Conductivity Ash in Raw Sugars; C-Ratio Method. General directions for making conductivity determinations have been given in Chapter XII (pp. 548–555). The simplest way to establish the relationship

[11] *Z. Rübenzuckerind.*, 14, 188 (1864).
[12] *Facts About Sugar*, 21, 1090 (1926).
[13] *Z. Ver. deut. Zucker-Ind.*, 75, 429 (1925).

between sulfated ash and electrical conductivity is to determine both in a number of samples, and to plot the results. If a smooth curve is obtained, this is then used to read off the ash content of other samples from the conductivity values. The quotient of the ash divided by the specific conductance is termed the "*C*-ratio."

The same concentration must always be maintained in the sugar solutions used for the conductivity determinations. For raw sugars, Lange[14] proposed to dissolve 5 g. to a total volume of 100 ml., because at this concentration the dissociation of the salts is near its maximum, and there is very close proportionality between the conductance and the sulfated ash. This concentration is used in Germany and has been adopted by Zerban and Sattler[15] in the United States. Some investigators have recommended higher concentrations, around 25 to 30 g. solids in 100 ml., since in this range the specific conductance reaches a maximum, and then falls off again owing to the increased depression of the conductance by the higher sugar concentration. In Czechoslovakia the normal-weight solution has been officially adopted, for obvious reasons. At this concentration the agreement between electrical and chemical ash values is not quite so good as when only 5 g. is used,[16] but Lazar[17] has shown that for practical purposes the choice of concentration between the limits of 5 and 26 g. is largely a matter of convenience.

Lange found that, at a concentration of 5 g. in 100 ml., the *C*-ratio of German beet sugars slowly increases with the ash content. Up to about 1 per cent of ash (sulfated ash less 10 per cent) the ratio is practically constant and equals 1786. The chemical ash can thus be calculated by multiplying the specific conductance by this figure. If, for example, the specific conductance is 0.000326, the ash content is $0.000326 \times 1786 = 0.582$ per cent.

The specific conductance is affected only by the salts present in solution, but not by those in the water-insoluble portion of the sugar. The chemical ash, as usually determined, represents the sum total in the water-soluble and the water-insoluble portions. This is one source of discrepancy between the two methods of determination. The sugar technologist is usually interested principally in the soluble part, and the conductivity method offers thus an additional advantage, besides speed and precision. In beet sugars the amount of insoluble matter is generally small, but cane sugars often contain considerable quantities of

[14] *Z. Ver. deut. Zucker-Ind.*, **60**, 359 (1910).
[15] *Facts About Sugar*, **21**, 1158 (1926).
[16] Spengler and Tödt, *Z. Ver. deut. Zucker-Ind.*, **78**, 1 (1928).
[17] *Z. Zuckerind. čechoslovak. Rep.*, **57**, 129 (1932/33).

suspended solids. For strict comparisons it is therefore necessary to use filtered solutions for the determination of both chemical ash and of conductance.

The *C*-ratio as a rule shows only slight fluctuations in raw sugars produced by the same factory or in the same district and, for beet sugars, in the same country. But the average *C*-ratio for the beet sugars of different countries is more divergent. Still wider variations are found in the *C*-ratios of raw cane sugars; for individual samples at a concentration of 5 g. in 100 ml., they range from about 1400 to over 2000, and the averages vary from 1560 for the British West Indies to 1802 for Santo Domingo. For this reason the simple *C*-ratio method has only limited application in the cane-sugar industry, being restricted to individual factories or small districts.

Determination of Conductivity Ash in Raw Cane Sugars by Two Conductance Measurements. Zerban and Sattler found[18] that the differences in the *C*-ratios of cane sugars, the solutions of which had been filtered, could not be ascribed to variations in the hydrogen-ion concentration or to differences in their sugar content and its depressing effect on the conductance. They are due principally to the nature and relative quantities of the salts present. Conductometric titrations with *N*/4 hydrochloric acid, according to Kolthoff's technique,[19] proved that sugars high in mobile inorganic anions, as Cl^- and SO_4^{--}, have a low *C*-ratio, while those high in organic anions of low mobility have a high one. Similar titrations with *N*/4 potassium hydroxide showed that the differences in the proportions of the cations are not large enough to affect the *C*-ratio. Conductivity determinations without and with addition of acid, on sugars of known ash content, made it possible to devise the following simple formula,

$$\text{Per cent soluble ash} = 0.0001757\ (9.13\ K + 1935 - K_1)$$

where "soluble" ash stands for the ash in the filtered solution, K is the specific conductance $\times 10^6$ of the sugar solution without acid, and K_1 that of the acidified solution, also multiplied by 10^6.

The method for determining the specific conductance in the presence of *N*/4 acid is described on p. 555. If the conductance of the solution indicates an ash content appreciably over 1 but not over 2 per cent, 2.5 g. of the raw sugar is used instead of 5 g., and 2.5 g. of ash-free sucrose is added for each 100 ml. of solution. Raw cane sugars with over 2 per cent of ash are rare, but if necessary even less than 2.5 g. may be used,

[18] *Facts About Sugar*, 21, 1158 (1926); 22, 990 (1927).
[19] Kolthoff, "Konduktometrische Titrationen," 1923.

and the difference between the normal quantity of 5 g. and the amount of sugar taken is made up by the addition of ash-free sucrose. The result is multiplied by the appropriate factor.

If the solutions are not filtered prior to the conductance determinations, the result of the measurement without acid, in terms of chemical ash, is affected by the water-insoluble matter, and that with acid by the salts insoluble in water but soluble in acid. In this case the following formula[20] may be used to calculate the total ash:

$$\text{Per cent total ash} = 0.001566\ K - 0.0001954\ K_1 + 0.4160$$

and the next to calculate the ash in the water-soluble portion of the sugar:

$$\text{Per cent soluble ash} = 0.001558\ K - 0.0002024\ K_1 + 0.4174$$

The result of the *C*-ratio method may, in the case of raw cane sugars, differ as much as 0.07 from the chemical ash. Conductance determinations on filtered solutions with and without acid reduce the maximum difference to 0.03, which is about the limit of error for the chemical method. The mean error is cut in half by the double procedure. But for raw beet sugars the latter method offers no advantages over the *C*-ratio method.

Conductivity Ash in Refined Soft and High-Ash Remelt Sugars. Soft refined sugars, at a concentration of 5 g. in 100 ml., have an average *C*-ratio of 1580, high-ash remelt sugars one of 1673. These ratios, compared to an average ratio of close to 1800 for raw sugars, indicate the removal of organic anions by the bone-black treatment. The ratios vary somewhat from refinery to refinery, and better agreement with chemical-ash figures is obtained by making two conductance determinations, without (K) and with (K_1) acid. The formula[21] for both soft and high-ash remelt sugars is

$$\text{Per cent soluble ash} = 0.0001695\ (9.13\ K + 1935 - K_1)$$

If the ash exceeds 1 per cent, the same procedure is used as described above for raw sugars.

Conductivity Ash in Hard Refined and Other White Sugars. The ash in this class of sugar is so low that it is preferable to use more than 5 g. in 100 ml. solution. Nees[22] recommended for granulated beet sugars a concentration of 25 g. in 100 ml., because the maximum con-

[20] Sattler, Mull, and Lorge, *Facts About Sugar*, 30, 377 (1935).
[21] Zerban and Sattler, *Ind. Eng. Chem., Anal. Ed.*, 3, 41 (1931).
[22] *Ind. Eng. Chem.*, 19, 225 (1927).

ductance is reached at about that point. The simple *C*-ratio method is sufficiently accurate for sugars of such high purity. For granulated beet sugars produced in the United States, at the concentration just given, the ratio equals 432 at 25° C., or 479.5 at 20° C. Refined hard cane sugars show a higher average ratio, 530 at 20° C.,[23] because they contain a lower proportion of mineral salts than beet sugars. The same figure applies also to refinery high remelt sugars containing up to 0.3 per cent of ash.

Water Correction for Refined Sugars. The sucrose present in the solution depresses the conductance not only of the salts in the sugar but also of the electrolytes in the.water used for dissolving it. When the ash content of the sugar is high compared to the electrolytes in the water, the depressing effect of the sucrose on the conductance of the water can be neglected. But when refined sugars containing very little ash are measured the conductance of the water often represents a large proportion of the conductance of the solution and sometimes may even exceed it. It is therefore necessary to correct the conductance of the water, to be deducted from the conductance of the sugar solution, for the depressing effect of the sucrose. Theoretically, this could be done by preparing a solution of perfectly ash-free sucrose and using its conductance as the water correction. But in practice it would be difficult to test for complete absence of ash in the sugar. However, the depressing effect of sucrose on the conductance of the water can be calculated by a method due to Arrhenius.[24] The latter found that the specific conductance of an aqueous salt solution κ_0 is related to the conductance κ of a solution to which p volume per cent of a non-electrolyte has been added, as shown in the following formula:

$$\kappa = \kappa_0 (1 - \alpha p/2)^2$$

where α is a constant whose value depends on the nature and concentration of the electrolytes and non-electrolytes. For sucrose and for the salts usually present, $\alpha =$ about 0.03. If a solution of 5 g. sugar in 100 ml. is used for the determination, the volume concentration equals 5/1.59 (the weight of the sugar divided by its specific gravity), or 3.15 per cent. By substituting these figures in the equation, it is found that the conductance of the water is reduced by the sucrose to 0.905 of its original value.

Arrhenius's equation was based on experiments with solutions containing not over 10 volume per cent of sucrose. But if it is assumed to hold also beyond this range, then 25 g. of sucrose in 100 ml., the

[23] Zerban and Sattler, *Ind. Eng. Chem., Anal. Ed.*, 3, 41 (1931).
[24] *Z. physik. Chem.*, 9, 509 (1892).

concentration advocated for determinations of the ash in refined sugars, reduces the conductance of the water to 0.6 of its value without dissolved sucrose. Hence the conductance of the water should be multiplied by 0.6 and the product deducted from the conductance of the solution of the sugar sample.

Water with a specific conductance of 0.0000015 to 0.000002, which may be readily obtained from efficient commercial stills, is adequate for ash determinations in most refined sugars. But if the conductance of the solution of the sample is about the same as that of the distilled water used, it becomes necessary to dissolve the sample in specially purified water.

According to Buse,[25] the specific conductance of ordinary distilled water, which may be as high as 0.00001, can be reduced to about 0.000001 by passing through it a rapid current of pure air, free from carbon dioxide. The air, which is aspirated by means of a vacuum pump, is first passed through a tower filled with soda lime and then through two tubes with glass wool. Distilled water purified in this way may be used for ash determinations in the highest grades of technical refined sugars. If the water, after the treatment with air, still shows a conductance higher than about 0.000002, it contains nonvolatile electrolytes and should not be used for ash determinations in refined sugars.

It goes without saying that the most scrupulous cleanliness is indispensable in conductance determinations on refined sugars. The flasks used should first be steamed out to remove all soluble constituents. They should not be closed with the thumb during the preparation of the solutions, but with rubber stoppers that have been washed with hot distilled water. The water used for dissolving the sugar must not be added from a wash bottle operated by mouth.

Conductivity Ash in Sirups and Molasses from Raw Cane Sugar Factories. The ash range in these sirups and molasses is about ten times as high as in raw cane sugars, from 1 to 4 per cent for sirups, and from 5 to 10 per cent or more for molasses. For this reason only 0.5 g. of material is taken for each 100 ml. of solution, and the sucrose deficiency is made up by the addition of 4.5 g. of practically ash-free sucrose. Conductance determinations are made without and with hydrochloric acid, and the molasses ash is calculated by the same formula as given above for raw sugars, the only difference being that the coefficient 0.0001757 is multiplied by 10 to correct for the tenfold dilution:[26]

$$\text{Per cent soluble ash} = 0.001757\ (9.13\ K + 1935 - K_1) \qquad (1)$$

[25] *Centr. Zuckerind.*, **44**, 780 (1936); **45**, 246 (1937).

[26] Zerban and Sattler, *Ind. Eng. Chem., Anal. Ed.*, **2**, 32 (1930).

For cane sirups, such as produced in Louisiana, the figure 1976 is substituted for 1935 in this formula.

Conductivity Ash in Refinery Sirups. The bone-black treatment which these products undergo removes organic anions. For this reason the coefficient in formula (1) is smaller and varies from one refinery to another. But a third conductance determination, in the presence of potassium hydroxide, makes it possible to use a formula applicable to all these products. Five milliliters of $N/4$ potassium hydroxide is added to 200 ml. of the original solution, and the conductance is measured as described on p. 555.

From the three conductance determinations the ash is calculated by the formula:[27]

$$\text{Per cent soluble ash} = 0.001757\ (13.3\ K + 4983 - 0.91\ K_1 - 5\ K_2) \quad (2)$$

where K_2 is the specific conductance times 10^6 of the solution containing potassium hydroxide.

General Formulas for Conductivity Ash in Sirups and Molasses from both Raw Sugar Factories and Refineries. If it is not known whether a sirup or molasses sample is a refinery or raw sugar factory product, the procedure must be further modified. Two different methods may be used:

(*a*) Method with three conductance determinations.[28] The same concentration is used as given previously, 0.5 g. product plus 4.5 g. ash-free sucrose in 100 ml. But normal phosphoric acid is substituted for $N/4$ hydrochloric acid, and the conductivity is measured without any addition, in the presence of potassium hydroxide and in the presence of normal phosphoric acid, as described on p. 555. The ash is calculated by the formula:

$$\text{Per cent soluble ash} = 0.0191369\ K - 0.002249\ K_2 - 0.00121\ K_3 + 3.07 \quad (3)$$

where K_3 is the specific conductance times 10^6 in the presence of phosphoric acid.

(*b*) Method with four conductance determinations.[29] In this method the addition of 4.5 g. ash-free sucrose is omitted, only 0.5 g. of the product being taken for each 100 ml. of solution. But this makes it necessary to carry out four conductance measurements, one without any addition, and the others in the presence of $N/4$ hydrochloric acid,

[27] Zerban, Sattler, and Lorge, *Ind. Eng. Chem., Anal. Ed.*, 2, 322 (1930).
[28] Zerban, Sattler, and Lorge, *Ind. Eng. Chem., Anal. Ed.*, 3, 38 (1931).
[29] Lorge, Sattler, and Zerban, *Ind. Eng. Chem., Anal. Ed.*, 4, 435 (1932).

N/4 potassium hydroxide, and *N* phosphoric acid, respectively. The following formula is used:

$$\text{Per cent soluble ash} = 0.01556\,K - 0.001125\,K_1 - 0.000623\,K_2 - 0.000219\,K_3 + 3.083 \qquad (4)$$

The reliability of the four formulas for the conductivity ash in sirups and molasses may be judged from the table below, showing the mean and maximum deviations from the chemical ash:

	Mean Error Per Cent	Maximum Error Per Cent
Equation 1 (Cuban and Puerto Rican blackstraps)	±0.145	±0.4
Equation 2 (Refinery sirups and molasses).....	±0.120	±0.3
Equation 3 (Raw and refinery sirups and molasses)	±0.136	±0.3
Equation 4 (Raw and refinery sirups and molasses)	±0.240	±0.3

When it is remembered that different analysts, using the same method for the determination of sulfated ash in molasses, may obtain results from 0.6 to 0.8 apart,[30] it is readily seen that the electrical method, with its much higher precision, is greatly superior to the chemical method, even for molasses.

Equations similar to those given above have been established by Davies[31] for Trinidad molasses, on the basis of conductivity determinations at 35° C.

Lever and Mazumder[32] have established a formula for the determination of *total* ash in molasses produced by both raw sugar houses and refineries, from a single conductance determination made at 20° C. upon a solution of 0.5 g. in 100 ml. In its final form it reads as follows:[33]

$$\text{Per cent total ash} = 0.013900\,K + 1.25$$

If the conductance determinations are made at any other temperature *t*, between 20 and 35° C., the following formula is used:

$$\text{Per cent total ash} = \{0.013900\,K\,[1 - 0.019\,(t - 20)]\} + 1.25$$

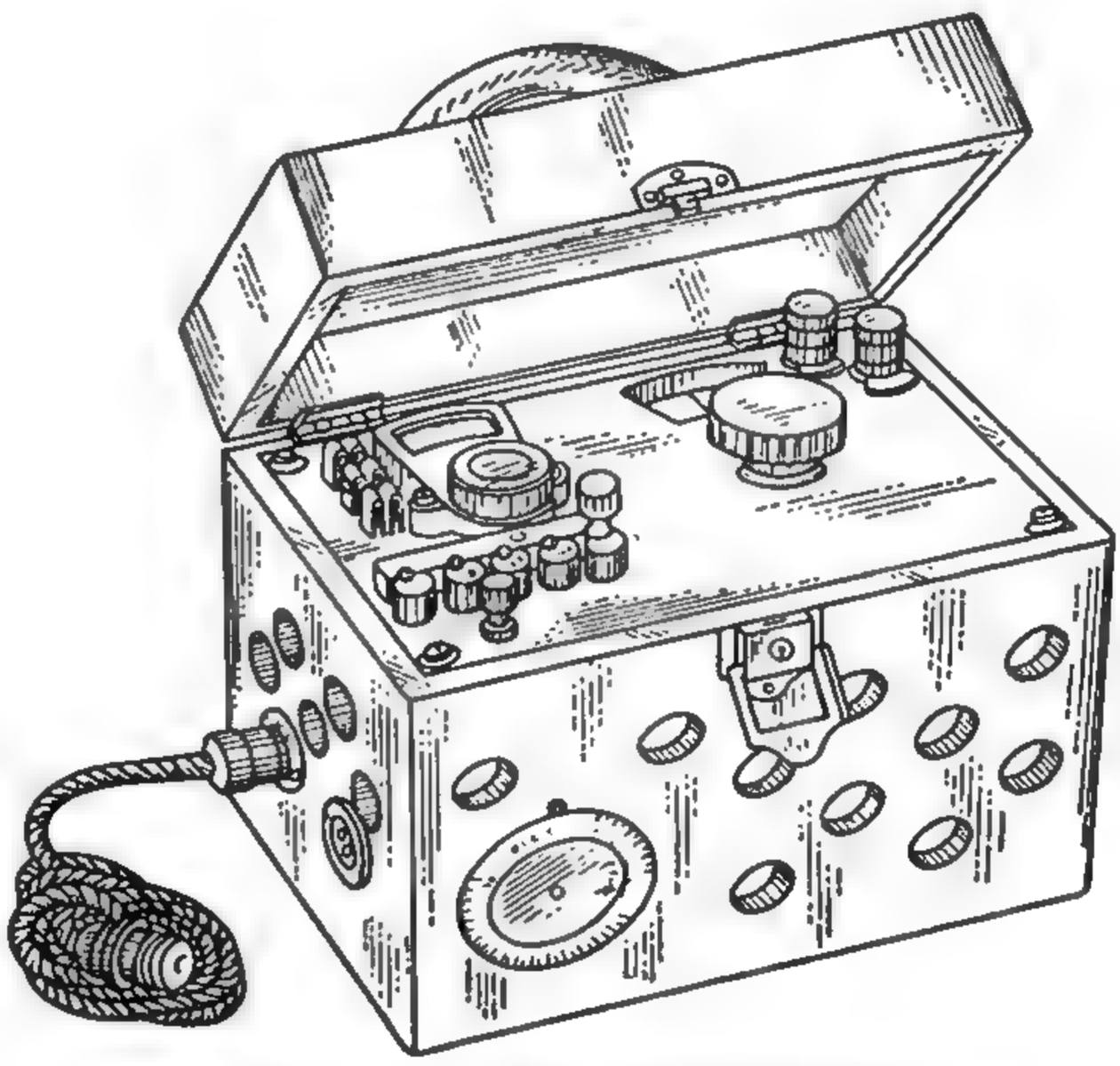
In view of the work of Zerban, Sattler, and Lorge, and of Davies, it is very doubtful whether these formulas are of such general applicability as claimed by Lever and Mazumder, except for rough approxima-

[30] Zerban, *J. Assoc. Official Agr. Chem.*, 4, 444 (1921).
[31] *Intern. Sugar J.*, 35, 472 (1933).
[32] *Intern. Sugar J.*, 38, 214 (1936).
[33] *Intern. Sugar J.*, 38, 303 (1936).

tions. According to Lever and Mazumder's own figures the deviations may amount to more than 1 per cent ash.

The general rule for conductivity ash determinations in all classes of sugar products is to use the simple *C*-ratio method wherever possible, and to resort to the more elaborate formulas only when actual comparisons with chemical ash figures prove this to be necessary. Calculations may be obviated by the use of nomograms.[34] Routine conductivity determinations are facilitated by the use of special instruments, some of which are described here.

The Sugar-Ash Bridge. Although the apparatus assembly described on p. 549 is well adapted for routine work, it has certain disadvantages also. The telephone is practically useless in a noisy factory laboratory; the necessary wiring is also an objection, because the connections must always be kept in good order. These inconveniences have been eliminated in several types of self-contained, compact instruments. One of these, the sugar-ash bridge,[35] is shown in Fig. 302. The fixed resistances, the slide wire, and a galvanometer are all enclosed in a case. The only outside connections necessary are one with a source of 110-volt alternating current, and another with the conductivity cell. On the front of the case are two concentric dials, one of which is set to the constant of the particular cell in use, and the other to the temperature of the solution in the cell. The slide-wire dial is calibrated in specific conductance. To determine the constant of the cell, the cell is filled with $N/50$ or $N/100$ potassium chloride solution and the slide wire set to the known specific conductance of the solution. The cell-constant dial is turned until the galvanometer reads 0 when the temperature dial is set to the temperature of the solution. The cell-



(Courtesy of Leeds and Northrup Co.)

FIG. 302. Leeds and Northrup sugar ash bridge.

[34] *Ind. Eng. Chem., Anal. Ed.*, 3, 38 (1931).

[35] Special catalog of Leeds & Northrup Co., Philadelphia.

constant dial is then locked in position. To determine the specific conductance of a solution, it is transferred to the cell and the slide-wire knob is turned until the galvanometer reads 0 while the temperature scale is set to the temperature of the solution. The specific conductance is then read off on the slide wire. Detailed instructions for the operation and maintenance of the instrument are furnished by the manufacturers.

If the conductance of a solution acidified with $N/4$ hydrochloric acid or N phosphoric acid is to be measured, the temperature dial settings must be changed, because the temperature coefficients of the acidified solutions are different from those of the original solution. The correct dial settings are shown in the following table:

Temperature of acidified solution, °C.	20.0	22.0	24.0	26.0	28.0	30.0
Correct dial setting, °C.	20.0	21.5	23.1	24.6	26.2	27.7

The Salometer.[36] This apparatus, of British manufacture, is very similar to the assembly used by Zerban and Sattler, but the dry cells, hummer, slide wire, and fixed resistance are all enclosed in a case. A pair of special telephone receivers is used to determine the null point. The cell is of the dipping type.

The Tödt-Gollnow Ash Meter. If conductance determinations are to be made only in aqueous solutions without further additions as is usually the case with beet products, the slide wire may conveniently be calibrated directly in per cent ash. This is a feature of the Tödt[37] apparatus, an improved model of which has been described by Gollnow.[38] Like the sugar-ash bridge, it requires only two outside connections, one with the line current and the other with the conductivity cell, which is of the Lange type but smaller and without water jacket. A galvanometer is used as null indicator. A concentration of 5 g. raw sugar in 100 ml. is used, and the per cent ash is read directly on two concentric scales, based on Lange's table, one for the range of 0.007 to 0.145 and the other for 0.145 to 3 per cent of ash. The temperature corrections are found from a table. The ash in beet molasses is determined by dissolving 5 g. to 500 ml. total volume, multiplying the ash value found on the scale by 5, and deducting 8 per cent from the result to correct for the smaller depressing effect of the reduced sugar concentration. Juices and intermediate products are dissolved to such a concentration that the ash percentage in the resulting solution falls within the range of the scale, and the result is multiplied by the dilution factor.

[36] *Intern. Sugar J.*, **34**, 150 (1932).
[37] *Z. Ver. deut. Zucker-Ind.*, **75**, 429 (1925).
[38] *Deut. Zuckerind.*, **59**, 708 (1934).

In order to obtain more accurate results upon low-grade beet sugars and upon beet molasses, Spengler, Zablinsky, and Wolf[39] have adopted the procedure, proposed by Zerban and Sattler, of diluting with pure ash-free sucrose in order to raise the sucrose concentration in the solution to about that of higher-grade beet raw sugars. Of low-grade beet sugars, 2.5 g. is weighed out, 2.5 g. of ash-free sucrose is added, and the mixture is dissolved to a volume of 100 ml.; the conductivity ash found is multiplied by 2. In the case of beet molasses, 0.5 g. of the molasses and 4.5 g. of ash-free sugar are dissolved to 100 ml., and the conductivity ash is multiplied by 10.

The " raffinometer " of Gollnow[40] is similar in design to the ash meter, but the two scales cover a range of 0.001 to 0.01, and 0.01 to 0.1 per cent ash; this instrument is suitable for ash determinations in refined and white consumption beet sugars. Another, universal model[41] is equipped with three scales, from 0.0015 to 0.015, 0.015 to 0.15, and 0.15 to 1.5 per cent ash; this covers practically the entire range of beet sugars, both refined and raw.

Conductometer of Šandera.[42] This instrument employs a somewhat different principle. The conductivity cell consists of a vertical glass tube, closed at the bottom and open at the top. One circular electrode is fixed horizontally near the bottom of the tube. The second electrode, of the same dimensions as the first, is mounted near the bottom of another glass tube, a little wider than the first and open at the bottom. This glass tube can be moved up and down over the other by a rack and pinion, and its exact position is indicated on a scale. The resistance of the solution in the lower tube varies in the same ratio as the distance between the electrodes. When the resistance exactly balances a fixed resistance, the ash content of the sample is read directly on the scale. The null point is indicated by a pair of electric-light bulbs which illuminate a field arranged and read like a polariscope field, but the instrument may also be equipped with a galvanometer, if desired.[43] The cell is filled through a side arm connected with a funnel, and emptied through another side arm with a rubber tube and pinchcock. The temperature correction is effected automatically by an arrangement similar to that in the sugar-ash bridge. The scale is calibrated directly in per cent ash, for a concentration of 26 g. of raw sugar in 100 ml. In the case of beet molasses, 1.35 g. is dissolved to 100 ml. total volume, and the ash percentage indicated on the scale is multiplied

[39] *Z. Ver. deut. Zucker-Ind.*, **88**, 691 (1938).
[40] *Centr. Zuckerind.*, **45**, 246 (1937).
[41] *Deut. Zuckerind.*, **63**, 292 (1938).
[42] *Z. Zuckerind. čechoslovak. Rep.*, **51**, 205, 603 (1926/27); **55**, 90 (1930/31).
[43] *Z. Zuckerind. čechoslovak. Rep.*, **60**, 290 (1935/36).

by 10. For ash determinations in intermediate products enough ash-free sugar is added to the solution to raise the polarization to about 100, and the result indicated by the scale is corrected for dilution. A special cell is used for finding ash in refined sugars. Tables and graphs for converting scale readings into specific conductance, or into ash, when other concentrations than 26 g. raw sugar or 1.35 g. molasses are used, have been published by Šandera.[44]

Determination of Organic Matter. The percentage of ash deducted from the percentage of total solids gives the percentage of organic matter.

Determination of Non-Sugar. The percentage of pol deducted from the percentage of gravity solids gives the percentage of non-sugar. The difference between gravity solids and sucrose is termed non-sucrose.

Determination of Organic Non-Sugar. The percentage of ash deducted from the percentage of non-sugar gives the percentage of organic non-sugar. The organic non-sucrose is calculated analogously from the ash and the non-sucrose.

Saline Quotient. This coefficient, also called pol-ash ratio, is found by dividing the percentage of pol by the percentage of ash. If the coefficient is based on sucrose, it is termed sucrose-ash ratio.

Reducing Sugar Ratio. The term glucose has been widely used in sugar-factory parlance to denote the mixture of reducing sugars containing principally dextrose and levulose. Since the word glucose, besides denoting the *d*-glucose of the organic chemist, may also mean unmixed corn sirup, the International Society of Sugar Cane Technologists has decided to do away with the existing confusion by calling the copper-reducing substances in sugar products reducing sugars, abbreviated R.S., and to use the words dextrose and levulose for *d*-glucose and *d*-fructose, respectively. The reducing sugar ratio represents the parts of reducing sugars per 100 of pol. The reducing sugar-sucrose ratio, or R.S.-sucrose ratio, expresses the reducing sugars per 100 of sucrose.

The determination of the R.S. ratio is of great importance in sugar-house control. Any increase in this coefficient during clarification or evaporation indicates a partial inversion of sucrose, while a decrease is due to the partial destruction of reducing sugars.

Determination of Extraction. The term extraction has also been given several meanings in consequence of which occasional confusion and misunderstandings have arisen.

The International Society of Sugar Cane Technologists has adopted the following terms:

[44] *Z. Zuckerind. čechoslovak. Rep.*, **55**, 221 (1930/31).

Juice Extraction. This denotes the weight of juice extracted by the mills, per 100 parts of cane. In each case it should be stated whether the weight of undiluted juice, juice diluted with imbibition water, or of mixed juice is referred to.

Pol Extraction. This is the pol in mixed juice per 100 parts of pol in cane.

Sucrose Extraction. By this is meant the sucrose in mixed juice per 100 parts of sucrose in cane.

When the term extraction is used without further qualification, the sucrose extraction is referred to.

Absolute Juice. The concept of "absolute juice," first introduced by Deerr, has been adopted by the International Society of Sugar Cane Technologists to replace the so-called normal juice which was meant to represent the juice as it exists in the cane. It is impossible to extract this juice, and its amount had to be calculated on the basis of various assumptions. These gave rise to many different interpretations of "normal juice." Absolute juice is simply the weight of the cane minus the weight of the dry fiber; the so-called adsorption water or colloidal water (p. 350) is thus included with the juice, and no arbitrary formulas are involved. The "undiluted juice" is defined as the juice expressed by the mills, or retained in the bagasse, corrected for imbibition water. For purposes of calculation it has the Brix of the primary juice, i.e., the juice expressed before any water is added.

Determination of the Acidity or Alkalinity of Sugar Products. The determination of the acidity or alkalinity of sugar products is at times a matter of considerable importance. The German official method for determining the acidity and alkalinity of raw beet sugars is selected for description.[45] The following solutions are used:

(1) Phenolphthalein. One part of phenolphthalein is dissolved in 30 parts of neutral 90 per cent alcohol. Two drops of this solution is used for every 100 ml. of solution. Commercial phenolphthalein is usually slightly acid, but neutralization of the indicator solution is omitted.

(2) Neutral Water. Ten liters of freshly boiled distilled water is mixed with 5 ml. of the phenolphthalein solution, and sufficient alkali (see under 4) is added to produce a permanent pink tinge. The water should be prepared several hours before use but should not be used after 1 or 2 days, as the pink color disappears during that time.

(3) Standard Sulfuric Acid. A $N/280$ sulfuric acid solution is pre-

[45] Frühling-Spengler, "Anleitung zu Untersuchungen in der Zuckerindustrie," 10th ed., p. 276, 1932.

pared by diluting 36 ml. *N* acid to 10 liters. One milliliter of this is equivalent to 0.0001 g. CaO.

(4) Standard Sodium Hydroxide. A *N*/280 sodium hydroxide solution is prepared, 1 ml. of which exactly neutralizes 1 ml. of the standard acid.

Ten grams of the sugar to be tested is weighed out. One hundred milliliters of the neutral water is placed in a porcelain dish and acidified by running in standard acid until the pink color just disappears. Then standard alkali is added drop by drop until the liquid becomes slightly pink again. The 10 g. of sugar is at once dissolved in the water. If the pink color is discharged the sugar is acid, and the acidity is measured by noting the volume of standard alkali necessary to restore the original color. If the pink tinge of the neutral water is reddened the sugar is alkaline and the alkalinity is measured by noting the volume of standard acid necessary to bring back the original tint. If the end point of the titration is over-run, the solution is titrated back with acid or alkali as the case may be. The acidity or alkalinity of the sugar is then expressed as the equivalent percentage of CaO. Thus 10 g. of a sugar requiring 30 ml. of standard acid for neutralization would have an alkalinity of 0.03 per cent CaO.

If the sugar is very dark, more than 100 ml. of water must be used. The solution must always be light enough so that the color change of the phenolphthalein may be observed without difficulty, but not more water should be used than is absolutely necessary. The titrations are greatly facilitated by the use of automatic burettes, provided with soda lime tubes. The operation must always be carried out in daylight or with a daylight lamp.

Accurate titrations of colored sugar solutions are greatly facilitated by the application of Walpole's compensation principle,[46] as proposed by Pall.[47] One of two test tubes, placed behind each other, is filled with water to which sufficient alkali has been added to produce the desired end point, and the other with the solution the acidity of which is to be determined. One of a second pair of tubes is filled with water, and the other behind it with the solution to be titrated. Standard alkali is added to this tube until the color of this pair of test tubes, viewed by transmitted light, matches that of the standard pair of tubes. For best results the light used for the observations is diffused with a frosted glass and filtered through a screen matching the color of the indicator at the end point.

Beet sugars which are acid in reaction are considered less suitable

[46] *Biochem. J.*, **5**, 207 (1910); see also p. **561**.
[47] *Can. J. Research*, **14** B, 299 (1936).

for refining and for storage, and such sugars are penalized in some countries in the calculation of the rendement (see p. 1040).

In Czechoslovakia beet raw sugars are tested qualitatively against phenolphthalein, and if the sugar is found to be acid, its reaction toward litmus is ascertained. The same two indicators are used for titrations with standard acid or alkali, but in commercial transactions the use of litmus is obligatory.[48]

The acidity or alkalinity of mill juice, clarified juice, etc., in the cane-sugar factory is frequently determined by titration with phenolphthalein, in a similar manner as described above. But this practice has been largely superseded by *p*H measurements (see Chapter XII), because the principal danger to be guarded against in sugar manufacture is inversion of the sucrose, and this is governed by the hydrogen-ion concentration rather than by the titratable acidity.

In beet-sugar manufacture the end point of the first saturation is usually also controlled by titration with phenolphthalein as indicator, but here the measurement of *p*H is likewise rapidly gaining ground. Saturation is continued until thymolphthalein paper shows the exact tint corresponding to the desired *p*H. The end point may be checked by colorimetric or electrometric *p*H determinations in the laboratory. Electrical conductivity measurements have also been proposed for the control of the first saturation.[49]

Determination of the Natural Residual Alkalinity. When beet or cane juice is treated with lime, the salts of acids which form insoluble calcium salts give a precipitate, and an equivalent quantity of sodium and potassium hydroxide is liberated. This alkalinity is termed the "natural" alkalinity. During the first saturation the free alkali hydroxides remain unaltered, but during the second saturation they are converted into carbonates which in turn react with lime salts. The natural alkalinity remaining is the "theoretical natural residual alkalinity." In practice the interaction between the alkali carbonates and the lime salts is not quantitative, however, and the natural alkalinity actually remaining is the "practical natural residual alkalinity." The juice of beets which have been grown or stored under adverse conditions contain considerable quantities of salts which are not precipitated by lime, and consequently the practical natural residual alkalinity is lower than for sound beets, and even the latter show considerable variation in this figure. The object of determining the practical natural

[48] *Z. Zuckerind. čechoslovak. Rep.*, 59, 41 (1934/35). For a discussion of the nature of the alkalinity of beet sugar, and methods for its determination, see Preininger, *ibid.*, 53, 617 (1928/29).

[49] Staněk and Šandera, *Z. Zuckerind. čechoslovak. Rep.*, 52, 209 (1927/28); Spengler and Tödt, *Z. Ver. deut. Zucker-Ind.*, 81, 1, 246 (1931).

residual alkalinity is therefore to ascertain whether a beet juice is normal, and what corrective measures should be taken to remove lime salts as far as possible.

Method of Spengler and Brendel.[50] The determination of the theoretical and practical natural residual alkalinity is carried out as follows: 500 ml. of juice from the first saturation is heated to boiling in an Erlenmeyer flask, filtered, and cooled (filtrate *A*).

One hundred milliliters of the filtrate is titrated with *N*/5 hydrochloric acid, phenolphthalein being used as indicator. The number of milliliters of acid used, multiplied by 0.0056, gives the total alkalinity due to lime plus alkali, expressed as per cent of CaO. Supposing that 18 ml. of acid is required for the titration, the total alkalinity equals 0.101 per cent CaO.

Another 10 ml. of filtrate *A* is placed in a shaking bottle, diluted to 100 ml., 3 drops of ammonia is added, and the mixture is titrated with soap solution (see p. 1075) to find the total lime. The corresponding grams of CaO are found from the table of Spengler and Brendel (p. 1076), and the result is multiplied by 10, to refer it to 100 ml. of original filtrate *A*. In the above example, 8.1 ml. of soap solution was used, which, corrected for 0.1 ml. of foam, gives 8.0 ml., corresponding to 0.0078 g. CaO in 10 ml., or 0.078 g. in 100 ml. of filtrate *A*.

The total lime, as CaO, is now subtracted from the total alkalinity, also expressed as CaO, and the result is the theoretical natural residual alkalinity: 0.101 — 0.078 = 0.023 per cent CaO.

To find the practical natural residual alkalinity, 300 ml. of filtrate *A* is neutralized with the required amount, in this instance $3 \times 18, = 54$ ml. of *N*/5 hydrochloric acid. The same quantity, 54 ml., of *N*/5 sodium carbonate solution, is added, the mixture heated to boiling in an Erlenmeyer flask under reflux, boiled for 3 minutes, filtered hot, and the filtrate is cooled (filtrate *B*). Two hundred milliliters of this filtrate is titrated with *N*/28 hydrochloric acid and phenolphthalein as indicator. In the above example 19.8 ml. was used; for the total amount of solution, $300 + 54 + 54 = 408$ ml., this corresponds to 40.4 ml. *N*/28. Since carbonate was titrated with phenolphthalein as indicator, and thus converted to bicarbonate, the result must be doubled: $40.4 \times 2 = 80.8$ ml., for 300 ml. of filtrate *A*. For 100 ml. the result is $80.8 \div 3 = 26.9$ ml. *N*/28 CaO, or 0.027 per cent practical natural residual alkalinity.

The residual lime is found by titrating 100 ml. of filtrate *B* with soap solution, and making the same calculation as described before. Sup-

[50] *Z. Ver. deut. Zucker-Ind.*, 77, 801 (1927); 78, 323 (1928); Frühling-Spengler, "Anleitung zu Untersuchungen in der Zuckerindustrie," 10th ed., pp. 225–227, 1932.

posing that 3.4 ml. of soap solution is required, the result, corrected for 0.1 ml. of foam, is 3.3 ml. = 3.0 mg. CaO. In the 408 ml. of solution, equivalent to 300 ml. of juice, there is $3 \times 4.08 = 12.24$ mg. CaO. In 100 ml. of juice there is $12.24 \div 3 = 4$ mg., or 0.004 per cent, CaO.

Method of Düwell and Solon. A simpler procedure for determining the practical natural residual alkalinity has been described by Düwell and Solon.[51] In this method 205 ml. of juice from the first saturation, measured hot at about 75° C., is transferred to an Erlenmeyer flask of 600-ml. capacity, and a few drops of 1 per cent phenolphthalein indicator are added. The juice is then treated with carbon dioxide gas at moderate temperature until the red color just disappears; if lime kiln gas is used it must be passed first through permanganate solution to remove hydrogen sulfide. Then 50 ml. of distilled water is added, the juice is boiled for 6 minutes and filtered through a rapid filter, and the precipitate is washed. The filtrate is transferred quantitatively to a 1-liter beaker, and the residual alkalinity is titrated with $N/5$ hydrochloric acid. Fifty milliliters of the neutralized juice is used to determine the lime content with soap solution. The volume of the remaining solution is ascertained with a measuring cylinder, and the residual lime in the original volume is calculated.

Since 205 ml. of hot juice, equivalent to 200 ml. of cold juice, was used for the titration with hydrochloric acid, the result must be divided by 2 to refer it to 100 ml., and must again be multiplied by 2 because titration with phenolphthalein as indicator gives only one-half of the alkalinity. The number of milliliters of $N/5$ hydrochloric acid used, multiplied by its CaO equivalent, 0.0056, therefore gives directly the practical natural residual alkalinity.

Determination of the Optimum Alkalinity. The optimum alkalinity of the final saturation is defined as that at which the juice contains a minimum of lime, that is, when neither free hydroxides nor bicarbonates are present.

Method of the Berlin Sugar Institute.[52] The standard method for determining the optimum alkalinity follows actual factory practice as closely as possible. About 1 liter of juice from the first saturation is heated to 90–95° C., and carbon dioxide is passed through it. Samples of 100 ml. each are removed at frequent intervals. Ten milliliters of each sample is titrated with $N/28$ hydrochloric acid, phenolphthalein being used as indicator. The remainder of each sample is heated to boiling and filtered, and the lime is determined in the filtrate by the soap method. For rapid tests it is sufficient to precipitate the lime as

[51] *Deut. Zuckerind.*, 54, 237 (1929).
[52] *Z. Ver. deut. Zucker-Ind.*, 78, 323 (1928).

oxalate, and to judge its quantity from the turbidity produced. That alkalinity at which the smallest amount of lime is found is the optimum alkalinity.

Use of Thymol Blue Paper. This is the simplest way to control the final saturation. It is only necessary to ascertain the exact tint which this paper gives when the standard method indicates optimum alkalinity, and to saturate to that tint. In place of this colorimetric *p*H determination, electrometric measurements of *p*H may be made by means of simple and inexpensive instruments.

Solon[53] has pointed out that theoretically the optimum alkalinity should be about one-half of the practical natural residual alkalinity, because in the determination of the latter the alkalinity is due to alkali carbonate, only half of which is indicated by the titration with phenolphthalein as indicator. But Spengler and Böttger[54] have shown that, although Solon's conclusion is correct for juices of normal natural alkalinity, the results of Spengler and Brendel's or of Düwell and Solon's method for determining the natural residual alkalinity, and thus of the optimum alkalinity, do not check with the standard method for determining the latter when the alkalinity is either high or low. This is due to the fact that the alkalinity is not entirely due to carbonates but partly to soluble alkaline salts of other weak acids acting as buffers.

Various efforts have been made to replace the standard method by more rapid procedures because at times the character of the juices worked in the factory changes rather abruptly. In all these rapid methods it is assumed that the minimum of lime salts is reached when neither fixed alkali nor bicarbonate is present in the juice. Spengler and Böttger[55] titrate two equal portions of the filtered saturated juice with standard hydrochloric acid, one with phenolphthalein and the other with methyl red as indicator. If both titrations give the same result the optimum alkalinity is reached. In Brukner's[56] method any bicarbonate present in the sample is first neutralized with a measured quantity of standard sodium hydroxide Then barium chloride solution is added with vigorous mechanical stirring, and the solution is titrated with standard hydrochloric acid and phenolphthalein. At the optimum alkalinity the amount of hydrochloric acid used is exactly equivalent to the sodium hydroxide added at the beginning. Spengler and Brendel[57] add calcium chloride solution to the hot juice. At the optimum alkalinity the addition of phenolphthalein indicator produces

[53] *Centr. Zuckerind.*, **36, 764 (1928).**
[54] *Z. Ver. deut. Zucker-Ind.*, **83, 11 (1933).**
[55] *Loc. cit.*
[56] *Deut. Zuckerind.*, **53, 1205 (1928) ; 62, 123, 284 (1937).**
[57] *Z. Ver. deut. Zucker-Ind.*, **79, 39 (1929).**

a muddy pink color; a yellow tint indicates oversaturation; a decidedly pink or red one undersaturation. It has been found in practice that the optimum alkalinity as determined by these three methods does not necessarily coincide with a minimum lime content. Studies by Dědek[58] have shown that the concentration of dissolved lime salts is actually determined by the concentration of CO_3^{--} ions, and that this in turn depends on the nature and quantity of the non-sugars.

Dutch Standard. The Dutch standard consists of a series of samples of cane sugar ranging in color from a very dark (No. 8) to an almost white (No. 25). These samples are put up each year in sealed bottles by two firms in Holland, under the direction of the Nederlandsche Handel Maatschappij (Netherlands Trading Company) in Amsterdam, and are sent to different parts of the world as color standards for classifying sugars, generally for the purpose of assessing duty. The relation between color and composition is such a loose one that the Dutch standard has purely an arbitrary value. Its use has been abandoned in all but a few countries, and duty is levied almost universally on the basis of polarization or of rendement (see p. 1040).

Color Standards for Soft Refined Sugars. These sugars are usually sold on the basis of color. It has been the custom of each refinery in the United States to use its own standards, consisting of actual soft sugars, graded by the eye, and ranging in color from a nearly white, No. 1, to a very dark, No. 15. The color of these sugars changes so rapidly, however, that the necessity of frequent replacements led to a search for more permanent standards. Powdered, colored glass was proposed for this purpose by Hooker, and this idea was further developed by Wills.[59] Four kinds of ground glass, colorless, yellow, light brown, and dark amber, are mixed in the proper proportions to duplicate the color of the soft sugars, enough glycerol being added to simulate the moist appearance of the sugar. The samples are put up in square bottles of colorless glass, stoppered, and sealed. An attempt was made to calibrate the standards by reflectance measurements with the spectrophotometer, but the values were found not to be reproducible.

Another method for preparing soft sugar standards has been described by Knowles.[60] He employs coarsely ground granulated sugar which is coated with a mixture of finely ground mineral pigments. The sugar thus colored must be darker than the darkest soft sugar. It is mixed in the required proportions with uncolored coarsely ground sugar, and enough Russian mineral oil is added to simulate the soft sugar.

[58] *Facts About Sugar*, 34, No. 11, 42; No. 12, 38 (1939).
[59] *Facts About Sugar*, 21, 1114 (1926).
[60] *Ind. Eng. Chem.*, 17, 980 (1925).

These artificial standards are fairly permanent, remaining practically unaltered for 6 months if protected from light.

Calculation of Rendement. This term is sometimes used as a synonym of available sugar or "basic recovery" (see below), but in the sugar trade it is generally taken to mean the quantity of refined sugar to be obtained from 100 parts of a raw sugar, and is used to determine the market value of the latter. The various formulas employed for its calculation subtract from the polarization, or sucrose, of the product a certain quantity which is taken to represent the melassigenic influence of the ash and other non-sugars. One of the most common methods of calculation is that first proposed by Monnier in France in 1863; Monnier assumed that 1 part of mineral impurities prevented the crystallization of 5 parts of sucrose, and so calculated the yield of crystallizable sugar by subtracting 5 times the percentage of ash from the polarization of the raw product. This method is very largely used in the valuation of raw beet sugars. If they contain raffinose, the per cent sucrose by the raffinose formula is used instead of the polarization. In some countries, such as Germany, 0.25 per cent is deducted from the rendement if the sugar is found to be acid according to the standard method (p. 1033). An invert-sugar content below 0.05 per cent is permissible, but if it is between 0.05 and 0.20 per cent, or in the case of second sugars between 0.05 and 0.50 per cent, 7 times its percentage is deducted from the rendement. Sugars in which the invert sugar surpasses these maxima may be rejected by the buyer.

For cane sugars, the following formula is often used: Rendement = Polarization — (5 × Per cent ash + 1 × Per cent invert sugar). In Australia this figure is referred to as the net titer. In Holland 5 times the ash and twice the invert sugar are deducted, in England 3 times the ash and twice the invert sugar.[61]

The number of methods used by different associations and factories for calculating rendement is almost unlimited.

Available Sugar. The formulas cited in the preceding section are purely arbitrary. For the purpose of technical accounting rather than commercial evaluation, it is necessary to know the solids and the sucrose content or purity of the raw material (juice, sirup, massecuite, raw sugar), of the sugar produced (raw or refined), and of the by-product (molasses or other run-off). In the beet-sugar industry the formula of Hulla-Suchomel,[62] or modifications of it, are widely used. If T represents the total solids in the raw material, T_1 those in the sugar made,

[61] Honig, *Arch. Suckerind.*, **37**, I, 635 (1929).

[62] *Z. Zuckerind. Böhmen*, **7**, 159 (1882/83); see also Paar, *Deut. Zuckerind.*, **64**, 517, 989, 1001, 1014, 1025 (1939).

and j, s, and m the purities of the raw material, sugar, and by-product, respectively, then

$$\text{Per cent available sugar} = \frac{100\ T\ (j - m)}{T_1\ (s - m)}$$

Deerr[63] has independently derived a more general formula, which may be used for either cane- or beet-sugar production:

$$\text{Per cent available sucrose} = \frac{100\ s\ (j - m)}{j\ (s - m)}$$

If refined sugar is produced, s equals 100. These formulas give correct results only if the purities refer to sucrose, not pol, and are based on solids by drying. However, Brix or refractometer solids may be used, provided that they are determined at equal concentrations of non-sugar.

Other formulas, which assume a fixed molasses purity, have been proposed by Winter, Prinsen Geerligs, and others.[64]

Determination of Crystal Content. The formulas previously discussed aim at the calculation of the total refined sugar that may be obtained from a given raw sugar, including the molasses film. But often it is desired to ascertain the quantity of solid sugar left upon removing the molasses film. Various methods have been proposed to determine this so-called crystal yield. In the oldest procedure, due to Payen,[65] the raw sugar was washed with 88 per cent alcohol which was saturated with sugar and acidified with acetic acid. The method was modified by Scheibler,[66] and later by Koydl,[67] who employed successive washings with saturated solutions of sugar in alcohol of increasing strength. The first two washing liquids were acidified with acetic acid, the next two had no acid added, and the final washing was accomplished with a saturated solution of sugar in absolute alcohol. It was found subsequently that in Koydl's method varying quantities of sucrose, salts, and coloring matter are precipitated from the molasses film. In order to avoid these errors, Herzfeld and Zimmermann[68] washed the sugar with a saturated aqueous solution of sucrose. This procedure has been further modified by Spengler and Brendel.[69]

[63] Deerr, "Cane Sugar," 2nd ed., pp. 438–440, 556–559, 1921.

[64] Prinsen Geerligs, "De Fabrikatie van Suiker uit Suikerriet," 4th ed., pp. 488–508, 1924. See also "Spencer's Handbook for Cane-Sugar Manufacturers," 7th ed., by Meade, pp. 359–362, 381–385, 1929.

[65] *Dinglers Polytech. J.*, 100, 127 (1846).

[66] *Stammer's Jahresber.*, Vols. 12 and 13 (1872 and 1873).

[67] *Oesterr.-ungar. Z. Zuckerind. Landw.*, 35, 277 (1906).

[68] *Z. Ver deut. Zucker-Ind.*, 62, 166 (1912).

[69] *Z. Ver. deut. Zucker-Ind.*, 77, 679 (1927).

Method of Spengler and Brendel. The entire operation is carried out at constant temperature, preferably at the standard of 20° C. The utensils must not be touched with the hand any more than is necessary, and it is advisable to cover the finger tips with rubber or asbestos. The concentration of the washing sirup is adjusted so that it is exactly saturated at the temperature at which it is to be used. The per cent by weight of sucrose in saturated solution at various temperatures is given in Table CXXXIV, which also shows the ratio of sugar to water.

TABLE CXXXIV

Temp. °C.	Per Cent Sucrose	Ratio, Sucrose to Water	Temp. °C.	Per Cent Sucrose	Ratio, Sucrose to Water
16	66.16	1.96	21	66.91	2.02
17	66.31	1.97	22	67.07	2.04
18	66.46	1.98	23	67.23	2.05
19	66.61	1.99	24	67.39	2.07
20	66.76	2.01	25	67.55	2.08

High-grade refined sugar is dissolved in a little less than half its weight of water at a temperature of about 60° C., and the solution poured hot into a bottle in which a few drops of formaldehyde have been placed previously as a preservative. The solution is thoroughly shaken, and polarized. The concentration is adjusted by adding the required quantity of water, and the polarization is checked again.

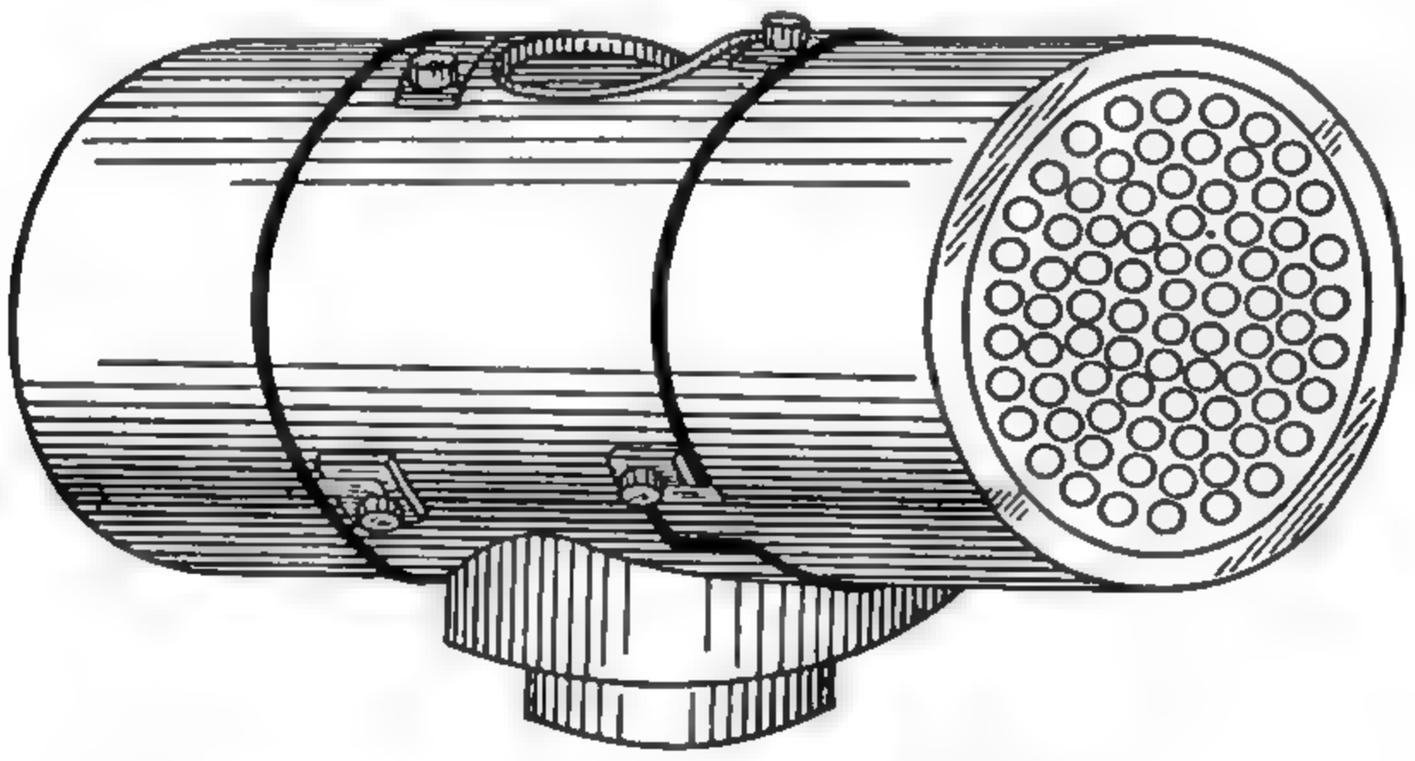


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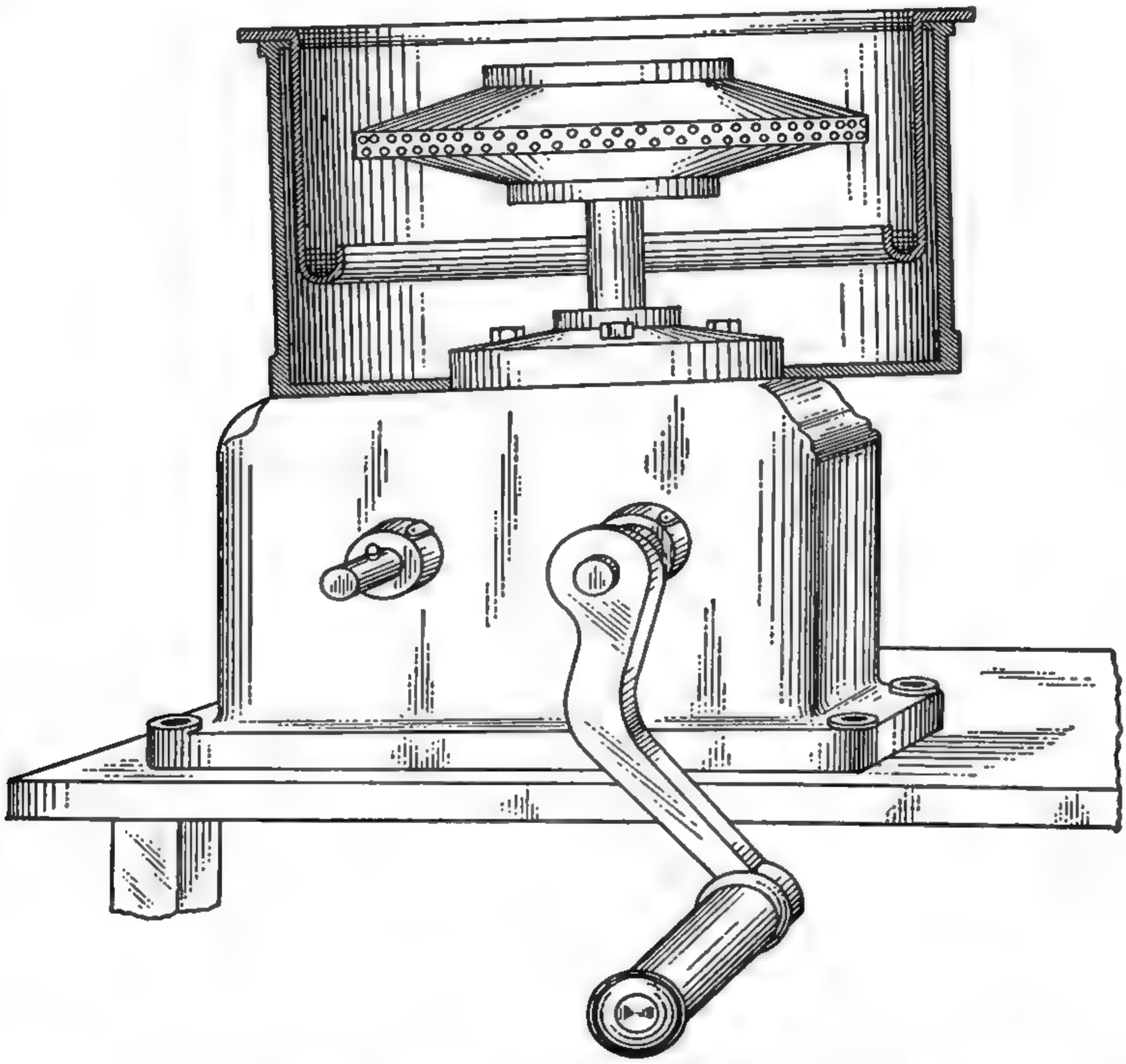
FIG. 303. Tubular centrifuge basket for determination of crystal content.

Fifty grams of the raw sugar is weighed into a sugar weighing dish, 50 ml. of the sugar sirup is added, and the mixture is stirred for 1 minute with a glass rod covered with rubber tubing. The mixture is allowed to stand for 2 minutes, and then stirred for 2 minutes longer. The mixing is carried out under a moistened cloth cover to prevent evaporation, and during the 2-minute rest period the dish is covered with a moist filter paper.

The crystals are separated from the mother liquor in the tubular centrifuge basket, Fig. 303, which fits on the spindle of the hand cen-

trifuge, Fig. 304.[70] The basket is prepared for the operation by removing the caps at both ends. A perforated metal disk is placed in the bottom of each and covered with a disk of metal gauze, and this in turn is covered with a disk of felt or flannel held in position by a



(Reproduced with permission from Z. Ver. deut. Zuckerind., 75, 626.)

FIG. 304. Centrifuge for determination of crystal content and of affining value of raw sugars.

metal ring. The caps are now fastened onto the center piece, the whole basket is dried for 1 hour in an oven at 103–107° C., and weighed. It is then placed in the constant-temperature room for 1 hour so that the felt or flannel may come to equilibrium with the moisture content of the atmosphere; otherwise it would adsorb water from the washing sirup.

[70] The centrifugal (No. 2413), and the basket (No. 2416), with directions for use and maintenance are furnished by E. Collatz & Co., Kesselstrasse 9, Berlin, N., Germany.

The basket is fastened to the spindle of the centrifuge, and the mixture of sugar and sirup is carefully poured into it in such a way that it is evenly distributed over both halves of the tube. The dish is set aside and covered with a moist filter paper. The basket is closed with a cork, and spun, with the 1 : 40 transmission of the centrifuge, for 1 minute at 75 revolutions of the handle per minute. The centrifuge is quickly stopped, the cork removed from the basket, and the remainder of the mixture in the sugar dish is washed into it with 25 ml. of washing sirup, in three successive portions. The cork is replaced, and the aluminum ring, furnished with the centrifuge, is placed over the rim of the shell. A moist cloth is spread over the opening and tied around the rim of the ring, to prevent evaporation. Spinning is now resumed at the same rate as before, for 3 minutes. The cork is removed, and the basket weighed. The perforated plates at the end of the basket are pushed in slightly in an oblique position, to allow free exit for the water vapor during the subsequent drying. The basket is dried for 2 hours at 103–107° C., allowed to stand for 1 hour in the air, and reweighed. The crystal content is calculated from the weights as shown in the following example:

Basket plus sugar, before drying	420.87 g.
Basket plus sugar, after drying	420.10 g.
Water	0.77 g.
Corresponding sugar, 0.77 × 2.01 (Table CXXXIV)	1.55 g.
Basket plus sugar, after drying	420.10 g.
Tare (basket plus felt or flannel)	372.08 g.
Total sugar	48.02 g.
Sugar from sirup film	1.55 g.
Crystals from 50 g. raw sugar	46.47 g.
Crystals from 100 g. raw sugar	92.94 per cent

Refined sugar, tested by this method, gave a crystal content of 99.90 to 100.09 per cent, and duplicate tests on raw sugars checked within 0.1 per cent.

Calculation of Composition and Purity of Molasses in Raw Sugars. A knowledge of the composition and purity of the molasses contained in raw sugars is often desired. The determination is made indirectly by subtracting the sucrose of the crystals from that of the raw sugar and calculating the remaining ingredients as due to molasses. If the raw sugar in the example given above had a polarization of 95.30, and a moisture content of 1.62 per cent, the purity of the molasses would be calculated as follows:

	Per Cent
Dry substance of raw sugar = 100 − 1.62	= 98.38
Crystal content of raw sugar	= 92.94
Difference = dry substance of molasses in raw sugar	= 5.44
Polarization of crystals	= 99.00
Polarization of raw sugar	= 95.30
Polarization due to crystals in raw sugar = 92.94 × 0.99	= 92.01
Difference = polarization due to molasses in raw sugar	= 3.29
Apparent purity of molasses in raw sugar = $\frac{3.29}{5.44} \times 100$	= 60.5

Refining Quality of Raw Sugars. The quality of a raw sugar, from the standpoint of refining, is determined not only by its chemical composition, but also by various physical characteristics which have an effect on refining operations. Foremost among these are the properties which affect: (1) the removal of the molasses film in the affination process, (2) the filtration of the melted sugar and of the wash (affination) sirup, and (3) the decolorization.

Affining Value of Raw Sugars. Two different principles are employed to estimate the extent to which the crystals may be purified in the affination process. In the method of Spengler and Brendel, and similar ones, the same general procedure is employed as for the determination of the crystal content. The Czechoslovakian method is based on the saline quotient of the washed crystals and of the mother liquor.

Method of Spengler and Brendel for Determining the Affining Value.[71] This method is carried out with the hand centrifuge mentioned on p. 1043, but equipped with another basket (No. 2418 with 760 perforations), shown in Fig. 304, which does not retain crystals of very small dimensions. The procedure thus approximates actual refinery operation. At the same time the purging quality of the raw sugar and the color of the washed sugar are also determined.

The washing sirup is prepared in the same way as for the estimation of the crystal content, but it should be saturated at a temperature about 3° C. below the working temperature, which must be kept fairly constant, near 20° C. Seventy-five grams of the raw sugar is transferred to the centrifugal basket, and 75 g. of washing sirup is weighed into a small beaker. The sirup is poured over the sugar, leaving a small quantity in the beaker, which is covered with a moist filter paper. The sirup is thoroughly mixed with the sugar by stirring for 4 minutes with a glass rod covered with rubber tubing. During this operation the sugar adhering to the walls of the basket is moved toward the center and evenly distributed around it. At the end of the 4 minutes the sugar

[71] *Z. Ver. deut. Zucker-Ind.*, 77, 229 (1927).

crystals on the stirring rod are washed into the basket with the sirup remaining in the beaker. The handle is placed on the shaft of the 1 : 20 transmission, and the drum rotated for exactly 20 seconds, with 20 revolutions of the handle. The centrifugal is quickly brought to a standstill, and the vessel in which the run-off collects is removed and weighed. The difference between this weight and the tare gives an approximate measure of the purging quality of the sugar, which is dependent mainly on the size and uniformity of the crystals. The sirup-collecting vessel is replaced by the splash plate, the basket is closed with a cork, the handle is transferred to the 1:40 transmission, and spinning is continued for 1 minute with 65 to 80 revolutions of the handle. The centrifuge is stopped, 25 g. of washing sirup is added, and centrifuging is continued for another minute and a half at the same rate as before. The cork is removed from the basket, and the basket is weighed with the wet sugar in it. The sugar is then evenly distributed over the bottom of the basket by means of a spatula, the crystals adhering to the spatula being removed with a pen knife. The basket is dried for 30 minutes in an oven at 103–107° C., being placed on a perforated sheet-metal plate. After cooling the basket is reweighed. The calculation is made in the same way as described previously for the crystal content (p. 1044). The result is expressed in parts dry, affined sugar, obtained from 100 parts of raw sugar.

In order to estimate the color of the affined sugar, this is transferred by means of a spatula to a piece of heavy wrapping paper, spread out, and pressed down with another piece of wrapping paper, without crushing the crystals. The sugar is again mixed on the paper, spread out, and pressed down once more. It is then placed in a sugar sample box lined with blue paper. This must also be done with a spatula, because pouring may separate the coarse from the fine crystals and change the appearance of the sugar. The surface of the sample is tamped lightly to make it even, and the color is compared, in diffuse light falling from above, with the standard samples of the Institute for Sugar Industry, Berlin.[72] There are five grades, No. 5 being the lightest. The color comparisons should be made independently by several observers, and the average taken.

Rapid Method of Spengler and Brendel.[73] In many instances the manufacturer is interested merely in the color of the affined sugar, and the following short method may be used for its estimation. The same apparatus is used as in the complete method described above. The 75 g. of raw sugar is mingled in the basket with 15 ml. of water for 3 min-

[72] *Z. Ver. deut. Zucker-Ind.*, 81, 693 (1931).
[73] *Z. Ver. deut. Zucker-Ind.*, 76, 801 (1926).

utes, at a temperature as close as possible to 20° C. The mixture is spun for 1 minute, with the 1:40 transmission, at 70 to 80 revolutions of the handle. The handle is removed, and while the centrifuge keeps on running, a special 5-ml. pipette is filled with water. The centrifuge is started up again, and the sugar is washed with the water from the pipette in such a way that it empties in 12 seconds, being moved up and down so as to wash the sugar evenly. Spinning is continued for another minute at the same rate as before. The centrifuge is then stopped, the sugar removed as described in the complete method, and dried in a thin layer in the air, or more quickly in a drying oven at a moderate temperature. The color is compared with the standards as described previously.

Horne's Washing Test.[74] Horne has objected to the use of water or of a saturated pure sugar sirup for affination tests, because the water dissolves some of the sugar, and the sirup tends to obscure the difference between high- and low-grade sugars through adding to each the same absolute, instead of the same relative, amount of pure sugar. He therefore prefers a saturated solution of the raw sugar itself.

One hundred grams of the raw sugar is mixed with 45 ml. of water. The mixture is shaken for 15 minutes, the sugar is allowed to settle, and 92 ml. of the resulting sirup is poured over 200 g. of the raw sugar and well mingled with it. The magma is purged in a 5-inch "Cyclone" laboratory centrifuge, which is brought to full speed in ½ minute. Spinning is continued for 2 minutes longer at 9 revolutions of the handle every 10 seconds. The basket is weighed, and the difference between this weight and that of the empty basket is divided by 2. The result is the percentage of wet affined sugar. If desired, the sugar may be removed from the basket and dried for 2 hours at 98° C, to obtain the percentage of dry affined sugar.

Affination Number of the Sugar Research Institute in Prague. The main purpose of the method of Spengler and Brendel, described above, is to ascertain whether a given raw sugar can be converted by simple affination into a consumption sugar of the desired color, and at the same time to estimate the probable yield and the amount of necessary washing. In contrast to this, the method used in Czechoslovakia, and originated by Šandera,[75] aims to evaluate a raw sugar from the standpoint of actual refining, which includes affination, decolorization, and crystallization. It is based on the idea that the rendement formula of Monnier (p. 1040) fails to consider the effect of affination on the distri-

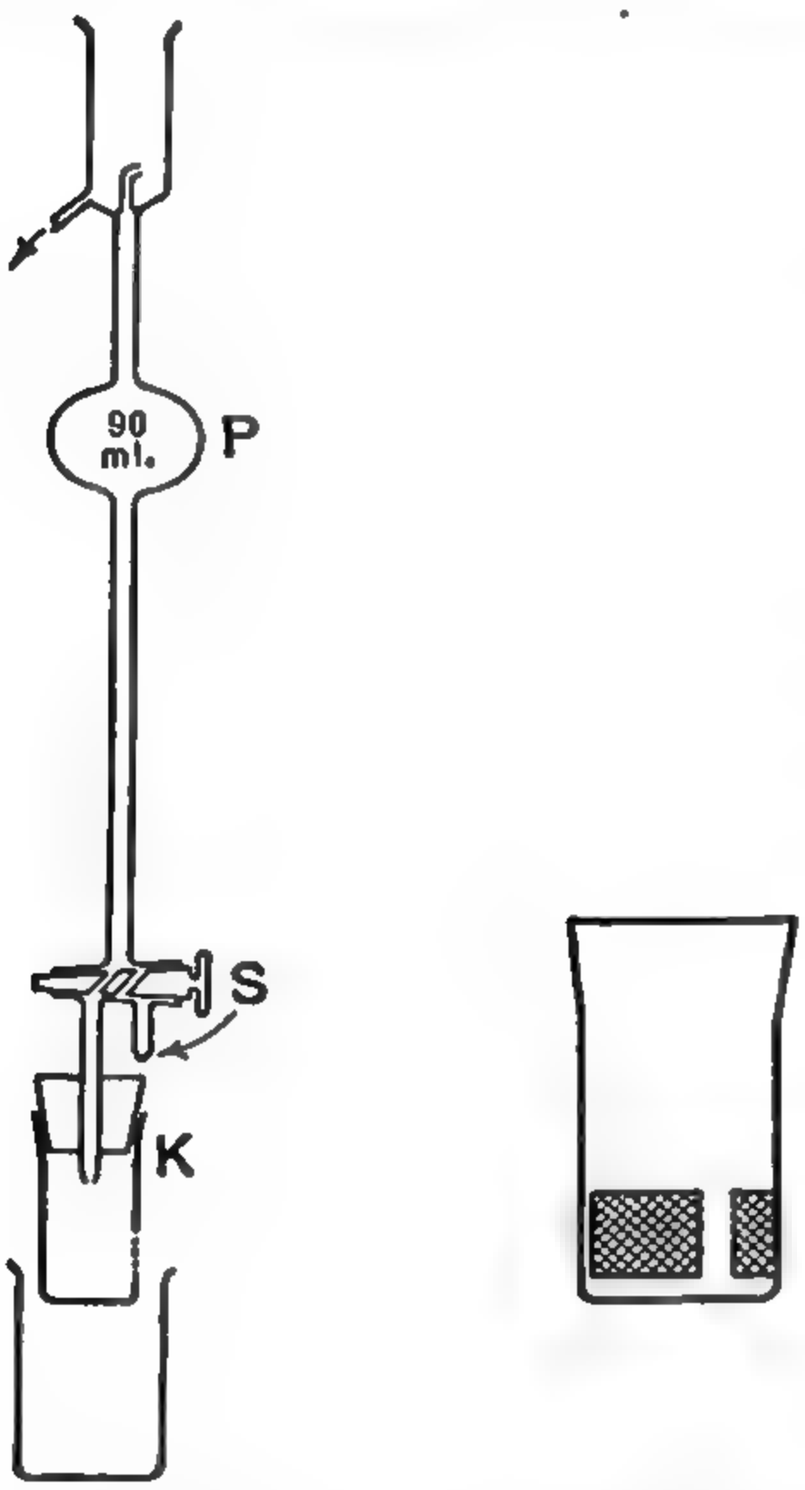
[74] *Ind. Eng. Chem.*, 10, 809 (1918).

[75] *Z. Zuckerind. čechoslovak. Rep.*, 52, 153 (1927/28); 56, 549 (1931/32); also private communication from Dr. Šandera.

bution of sugar and ash between the washed sugar and the affination sirup. At the same time the conductometric determination of ash is introduced, because the chemical ash includes insoluble ash which has no effect on the rendement. The sugar is washed with water, and the conductivity ash and the polarization or refractometer solids are determined in the washings and in the washed sugar. The affining quality of the sugar is the higher, the less sugar and the more ash are removed by washing. This relationship may be expressed by the formula:

$$\text{Affination number } (AN) = \frac{p_1 \times v_2}{p_2 \times v_1}$$

where p_1 and v_1 are the polarization and conductivity, respectively, of the washings, and p_2 and v_2 those of the crystals, after being dissolved in water. Instead of the polarization, the refractometer solids may be used without materially affecting the result.



(*Courtesy of Dr. K. Šandera.*)

FIG. 305. Apparatus for determining affination number.

The apparatus used for the determination is shown in Fig. 305. *P* is an automatic pipette of 90-ml. capacity. It is filled with distilled water from a storage bottle through the short nipple *S* below the three-way stopcock, and the excess runs off through the overflow funnel on top of the pipette. When the stopcock is turned to the position shown in the figure, the pipette empties in 30 ± 1 seconds into a small vessel *K* with metal gauze insertions, acting as a strainer. The whole apparatus is placed over an ordinary beaker to receive the washings. The equipment is standardized, and is furnished with a certificate of the Sugar Research Institute. The outflow time of the pipette should be checked from time to time, and it is advisable to calibrate the apparatus occasionally with a sugar of known affination number.

The pipette must be thoroughly cleansed with chromic acid mixture before use. Fat must not be used for lubricating the stopcock. After each test the strainer is rinsed with dilute ammonia (1 : 5), followed by washing with water. Before each use it is immersed in distilled water for 10 minutes.

Fifteen grams of the sugar is weighed out in a tared capsule, and poured quantitatively into the strainer, which has been dried imme-

diately before by wiping with a clean cloth. The sugar is thoroughly mixed in the strainer by stirring with a heavy wire, and the surface is evened with a brass cylinder, without exerting heavy pressure. The strainer is fastened to the rubber stopper around the lower end of the pipette in such a way that it is always in the same position, which may be marked on the stopper with a pencil. The tip of the pipette must be well centered over the strainer. The pipette is filled with water, and then emptied over the sugar by turning the stopcock. The washings are allowed to drip for 10 seconds, after which the beaker is removed and its contents are well mixed. The temperature during the entire operation should vary not more than 1° from an average temperature of 20° C. The conductance of the washings (v_1) is determined in the conductometer of Šandera (p. 1031), and expressed directly in scale divisions of the instrument without further calculation. The remainder of the washings is clarified with dry lead subacetate, and the direct polarization (p_1) is read in a 200-mm. tube. The sugar left in the basket is dissolved in water, and the solution diluted to 100 ml. in a sugar flask. The conductance and the polarization are determined in the same way as described for the washings. This gives v_2 and p_2. The affination number, which is designated by the symbol $AN_{p'}$, when measured by this method, is then calculated by the formula given above.

If the refractometer solids are determined instead of the polarization, r_1 and r_2 are substituted for p_1 and p_2, respectively, in the formula.

Simplified Method of Šandera and Mirčev.[76] The method just described requires four separate measurements. The procedure may be materially shortened by the use of an empirical formula which is based on the conductance and polarization or refractometer reading of the washings only, and the conductance (v) and polarization (p) of the original sugar, which are usually determined as a routine matter. It is therefore not necessary to recover the washed sugar, but the strainer is simply cleaned and is ready for the next determination. The formulas derived by Šandera[77] for this modified method are:

$$AN_p = \frac{p_1\,(v - 0.9\,v_1)}{0.9\,v_1\,(0.72\,p - p_1)} \quad \text{(based on polarization)}$$

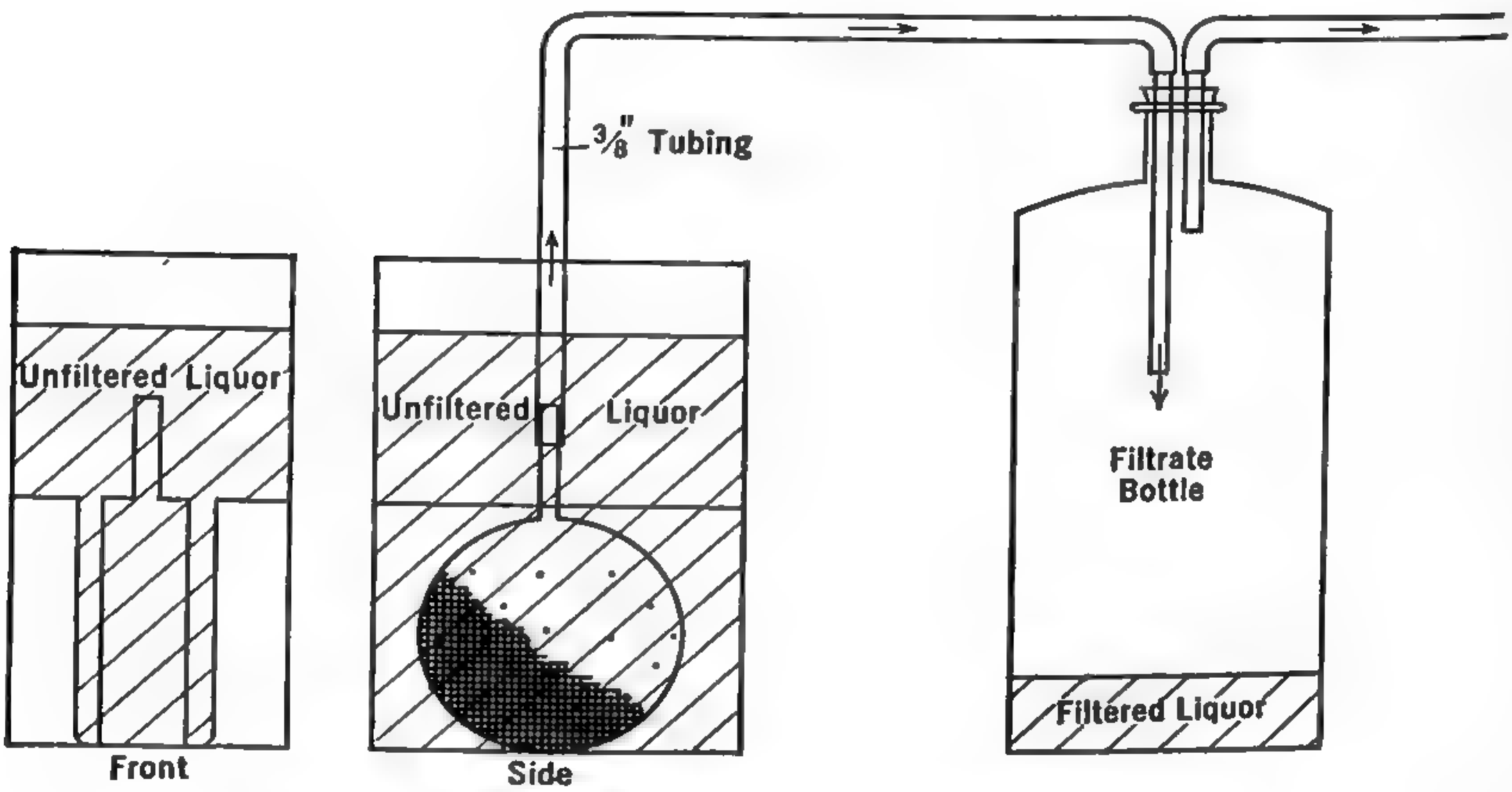
$$AN_p = \frac{r_1\,(v - 0.9\,v_1)}{0.9\,v_1\,(0.19\,p - r_1)} \quad \text{(based on refractometer solids)}$$

The values of AN_p obtained by the simplified method are different from those of $AN_{p'}$ found by the original procedure, and for this reason the method used must be specified.

[76] *Z. Zuckerind. čechoslovak. Rep.*, **58**, 357 (1933/34).
[77] *Z. Zuckerind. čechoslovak. Rep.*, **56**, 549 (1931/32).

The affination number varies inversely with the affining quality of the sugar, and AN_p is about 0.4 for the normal beet sugar produced in Czechoslovakia. Šandera has proposed to correct the rendement, calculated according to Monnier (p. 1040), by making a deduction when the affination number is above 0.4, and vice versa. If the uncorrected rendement is, e.g., 90.80, and the affination number 0.53, then 0.53 — 0.40, or 0.13, is deducted from the rendement, resulting in a corrected rendement of 90.67.

Filterability of Raw Sugars. Raw sugars always contain varying quantities of colloidal matter, which slows up filtration in the refinery, no matter what clarifying agents are used. This retarding effect may be measured in the laboratory by filtration experiments, or the quantity of colloids may be determined by special methods.



(Reproduced with permission from Facts About Sugar, 20, 768.)

FIG. 306. Diagram of Elliott filtration apparatus.

The laboratory filtration methods may be divided into two general classes, those which employ vacuum filtration at or near room temperature, and those which employ pressure filtration at the temperature used in refinery operation, about 180° F.

Elliott Filtration Method. The most widely used vacuum filtration apparatus is that designed by Elliott and described by Blowski[78] (Fig. 306).[79] The filtering element is a leaf of circular cross section, with two filtering surfaces of coarse mesh screen, 4 inches in diameter, and

[78] *Facts About Sugar*, 20, 758 (1925).

[79] Manufactured by Eimer & Amend, Third Ave. and 18th Street, New York, N. Y.

covered with filter cloth. The leaf is placed in the lower compartment of a vessel for the solution to be filtered, and is connected by pressure tubing with a large glass bottle in which the filtrate is collected and weighed. The outlet on the bottle leads to the vacuum pump.

Since the method is purely empirical, the make of filter cloth, the kind and amount of filter aid, and the vacuum and temperature must be standardized and strictly adhered to. The Special Committee on Uniformity in Reporting Factory Data, of the International Society of Sugar Cane Technologists, has recommended[80] the filter cloth sold under the trade name " M-451, Calcot," specified as 2-yard, 40-inch drill, 70 by 84 weave, furnished by the California Cotton Mills, Oakland, California. The filter aid to be used is the " Filter-Cel Laboratory Standard," specially prepared by the Johns-Manville Corporation, Manville, N. J. A temperature of 27.5° C. has been chosen as the standard, because the 20° C. specified by Blowski is difficult to maintain in the tropics. A vacuum of 26 inches at sea level, or 4 inches of absolute pressure, is employed. If all these standards are carefully adhered to, a standard sugar is not necessary. It would be a very difficult matter to store and distribute to all those using the apparatus a sufficient quantity of such a sugar. Refined sugars produced by different refineries show different filtration rates, and are therefore also unsuitable as a standard. This applies even to medium granulated sugar.

The filter cloth is cut into disks, 8 inches in diameter. One disk each is placed over the two faces of the filter leaf, with the ribbed side out. Dry, new cloths must be used for each test. The cloth is fastened with a piece of soft iron wire, wound around behind the projecting ring on the edge of the leaf, and fastened by twisting it with a pair of pliers. A second piece of wire is wound next to the first, but the loop is closed at a point 180° from the first loop. This prevents the solution from passing by, instead of through, the cloth. Before tightening the loops, the cloth is pulled evenly over the screen, without stretching. It is then trimmed off. The prepared filter leaf is placed in the filtration vessel, correctly centered, and connected with the collecting bottle and the vacuum line. Care must be taken that all connections are absolutely air tight.

A 1500-g. sample of the sugar is dissolved in 1500 g. of distilled water at room temperature, preferably by means of a mechanical stirrer. With sugars of high filtration rate it may be necessary to use 1800 or 2000 g. each of sugar and water. Two per cent of the filter aid, calculated on the weight of the sugar, is added to the solution, and allowed to sink in by its own weight. It is then well mixed with the sirup with

[80] *Facts About Sugar*, 29, 7 (1934).

a spoon or mechanical stirrer, and the temperature is adjusted to 27.5° C.

The mixture is rapidly poured through a flour sieve into the filtering vessel, and allowed to stand for exactly 2 minutes by the stop watch. The vacuum valve is opened, and filtration is carried on for exactly 30 minutes at 26 inches vacuum, which must be reached in 2 minutes from the beginning of the filtration. The vacuum must be kept constant during the remaining 28 minutes by either hand or automatic regulation. The temperature is also maintained at 27.5° C. during the entire operation, and the filtering vessel may be placed in a large water bath for this purpose. At the end of the 30 minutes the vacuum is quickly broken, and the amount of filtrate collected in the bottle is determined by weighing.

A quantity of 3000 g. of filtrate has been arbitrarily chosen to represent a filtration rate of 100. The filtration rate is thus calculated by dividing the weight of filtrate by 30. The result is designated as the "Revised Elliott Filtration Rate."

Several filtrations may be carried out simultaneously by connecting each apparatus with a vacuum manifold, in battery fashion.

The maximum difference between duplicate determinations is about 6 per cent. Nevertheless, the test has proved very valuable in practice by serving as a guide in efforts to improve the filtering qualities of raw sugars. A high correlation between the results of laboratory tests and refinery operation, however, cannot be expected, for several reasons. In the refinery the liquors are always limed to a certain *p*H, but this is omitted in the laboratory tests, in order not to complicate them. In large-scale operation there are many uncontrollable or uncontrolled factors, such as prolonged heating or standing of the liquors, incipient fermentation, reuse of filter cloths, etc., all of which affect the filtration rate. Furthermore, the filtration rate varies with the amount of filter aid used, and in reality the tests should be made with varying amounts of filter aid, and a curve plotted. But such a procedure would not be practical for routine work, and it has also been shown that 2 per cent of filter aid in the laboratory test is equivalent to the economic optimum in the refinery.

Kopfler[81] has found that the precision of the Elliott test may be materially increased by substituting Monel metal screen (20 × 120 mesh) for the cotton cloth. The metal cloth is thoroughly washed after each filtration, and used over and over again without impairment of its filtering capacity. The results obtained by this method are not directly comparable with those of the revised Elliott filtration test, be-

[81] Private communication.

cause the dimensions of the filtering vessel must be changed to accommodate the filter leaf with metal screen.

Retardation Factor. This figure is used to compare the filtration rates of sugars of varying polarization. It is calculated by the formula:

$$\text{Retardation factor} = \frac{135 - \text{Filtration rate}}{100 - \text{Polarization}}$$

When the revised Elliott filtration rate is used to calculate this factor, it is termed the "revised retardation factor."

Pressure Filtration Test for Raw Sugars. The principal objection to the Elliott test is that it is carried out at room temperature, while the refinery operates at about 180° F. A number of different apparatus have been used to measure the filtration rate of sugars at this temperature. The one described here is of comparatively simple construction and was designed in the research laboratories of the Johns-Manville Corporation.[82] A picture of the complete equipment is shown in Fig. 307, and two views of the filter element in Fig. 308. As a convenient and cheap source of pressure a cylinder of compressed nitrogen (97 per cent N), with a reducing valve, is used, but a compressor may be substituted if preferred. The gas enters the filter chamber through a perforated pipe ring near the bottom of the chamber; a baffle plate is mounted between the pipe and the filter element. The filtering surface is circular, 1.5 inches in diameter. The cloth is backed by a coarse wire screen and held in place by a threaded cover. The filter chamber is maintained at the required temperature by means of an ordinary Bunsen burner. The temperature is measured with a thermometer which is placed in the well mounted next to the filter element in such a way that the bulb is in line with the center, and back of the filter element. A portion of the gas entering the filter chamber is used for stirring and is allowed to escape. As the rate of settling of the filter aid and the colloidal properties of the solution are affected by the degree of agitation, the flow of the gas must be standardized. Its rate of flow is measured with a flow meter[83] calibrated in milliliters of nitrogen passing per minute. The pipe leading from the filter chamber to the flow meter is bent at right angles six times to prevent clogging of the capillary by entrained particles.

The same standard Filter-Cel and standard cloth are used as in the revised Elliott test. A fresh, dry cloth circle is cut for each test. When the cover is screwed on, care must be taken that the inside edge

[82] *Facts About Sugar*, 29, 7 (1934).

[83] Furnished by Pardee Engineering Co., Long Island City, N. Y.

of the rubber washer is flush with the inside edge of the cover so that the filtering surface has a diameter of exactly 1.5 inches.

A 60-Brix solution of the sugar to be tested is prepared by mixing 1800 g. of sugar (or a little more, depending on the moisture content of the sugar) with 1200 g. of water in an enamelware bucket. The sugar is dissolved by stirring with a mechanical stirrer. Then 0.7 per

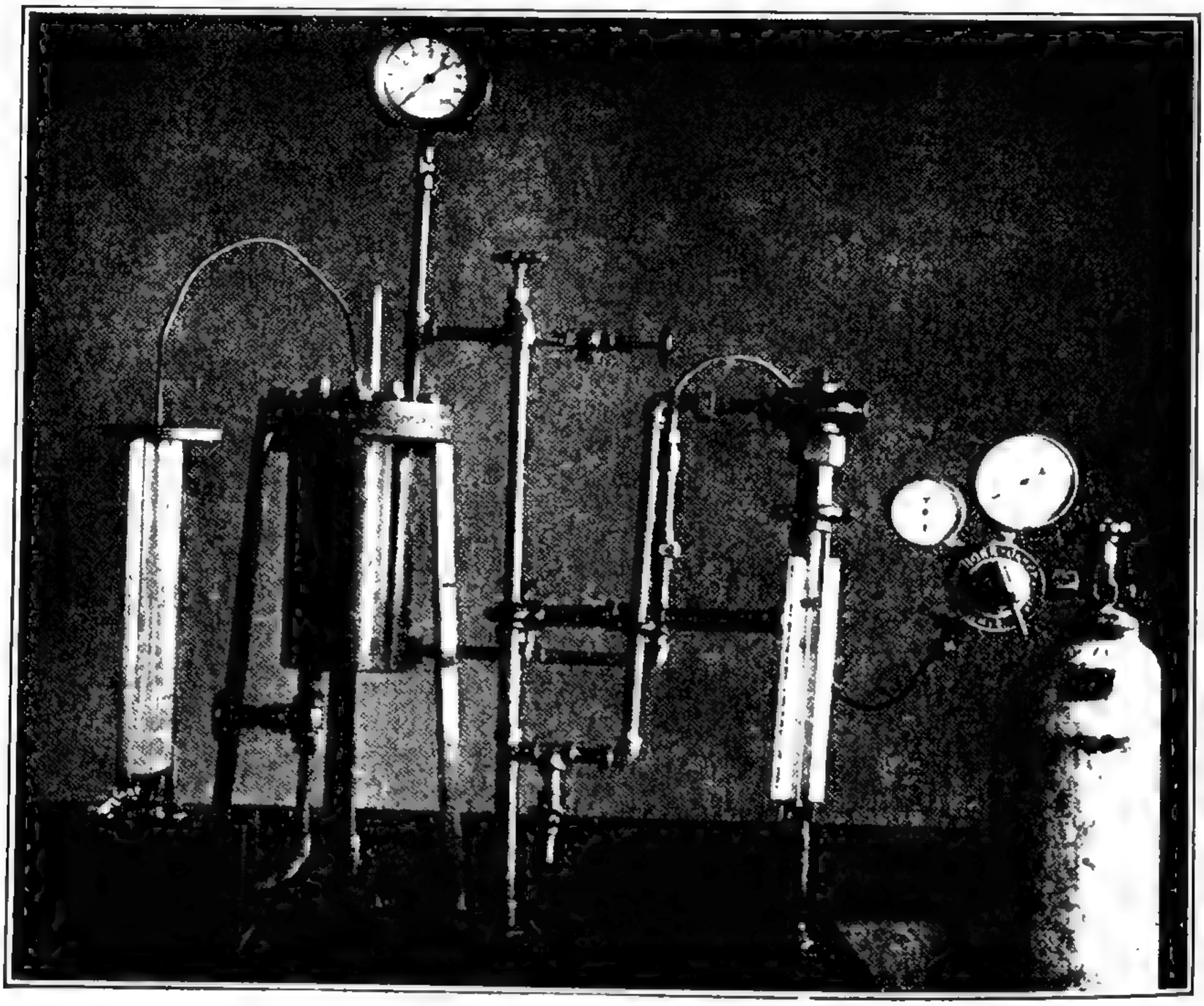


FIG. 307. Pressure filtration apparatus for determining filterability of raw sugars.

cent of standard Filter-Cel on the weight of the sugar is added and allowed to sink in by its own weight, and stirring is continued for 5 minutes longer. The solution is heated to 180° F., and at the same time water is heated in the filter chamber to the same temperature by means of the Bunsen burner. The water is emptied out; the chamber is rinsed twice with the sugar solution and then filled about three-quarters full. At the same time some gas is passed through to keep the Filter-Cel from settling. The filter element is inserted and clamped tight, the temperature is adjusted to exactly 180° F., a beaker is placed under the filter outlet, and the pressure is built up to 10 pounds in 15 seconds.

It is held at that figure for 20 seconds longer, and at the same time the current of the nitrogen gas is regulated so that 50 ml. of it passes per minute. At the end of the 20 seconds the beaker is replaced by a previously weighed 500-ml. cylinder. The cylinder is covered by a plate with a hole in the center, through which the delivery tube for the filtrate is inserted. The temperature, pressure, and gas flow are now held constant for 10 minutes by the stop watch. The pressure is then gradually raised to 30 pounds within 15 seconds, and at the same

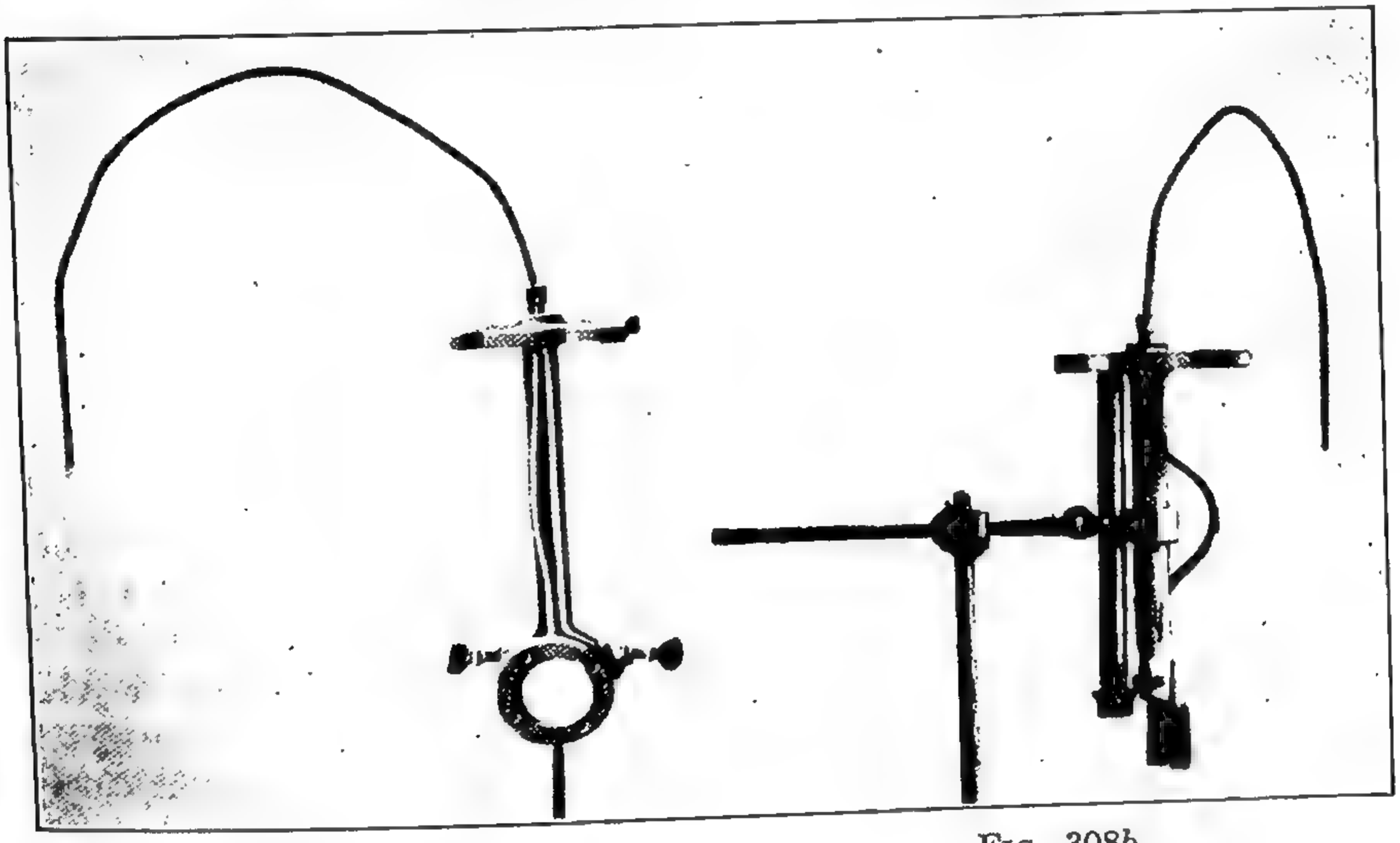


FIG. 308*a*.
Front view

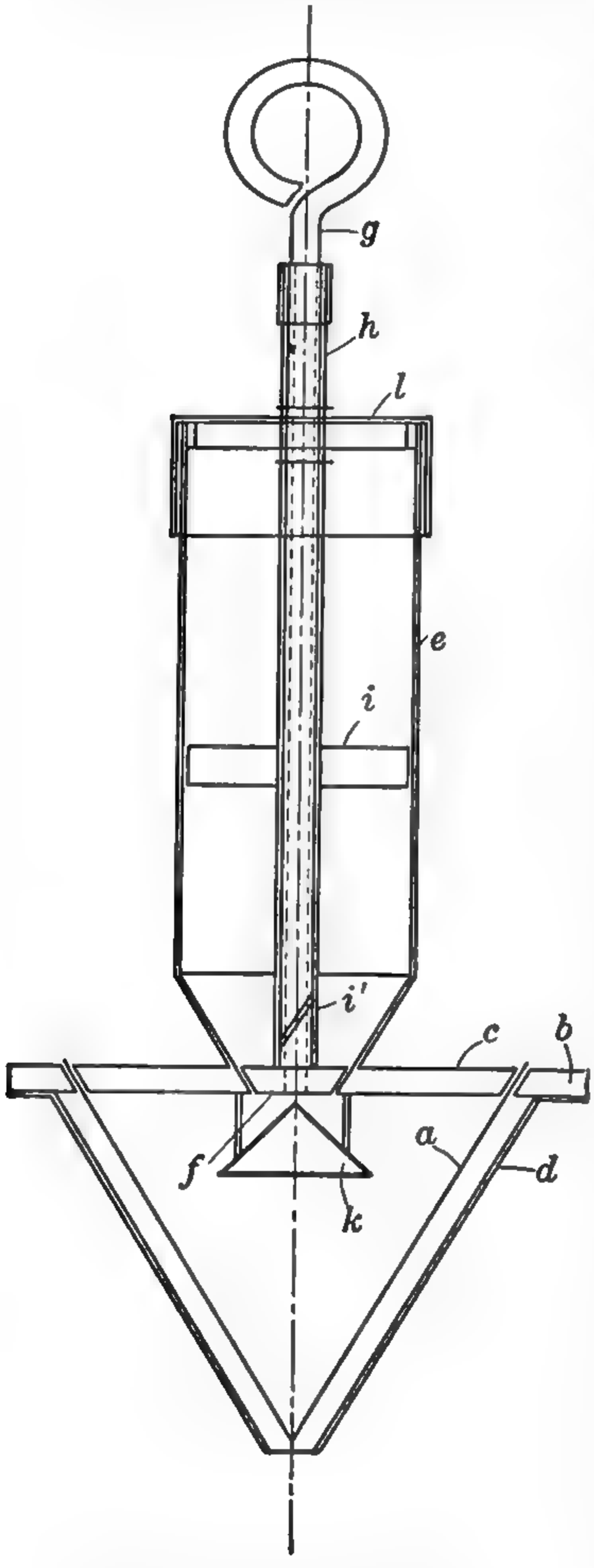
FIG. 308*b*.
Side view

of filtering element for pressure filtration apparatus.

time the gas flow is adjusted to 150 ml. per minute. The pressure is held at 30 pounds until the total filtration time is 30 minutes from the moment the cylinder was put into place. The cylinder is removed and weighed, and the amount of filtrate is found by difference. When the filtrate has cooled sufficiently its Brix is determined by refractometer, and the quantity of total solids filtered is calculated. If a graduated cylinder is used, a time-flow diagram may also be prepared.

The pressure tests do not check any better with large-scale factory operation than does the Elliott test, for reasons already given in the discussion of the latter. For routine laboratory work the Elliott test is preferable because of its simplicity and the lower cost of the equipment. But the pressure method offers greater precision and is better adapted for investigations on filtration rates under varying conditions.

Filterability of Juices. The filtration rate is also an excellent criterion for judging the quality of beet juices, and the efficiency of various methods of liming and saturation. The apparatus designed for its determination by Spengler, Tödt, and Böttger[84] is illustrated in Fig. 309. It is constructed of brass. The paper filter *a* is held fast at its upper edge between the concentric covers *b* and *c*. It is surrounded by a jacket *d*, for the purpose of heat insulation, but must not touch it at any point, hanging perfectly free. The juice to be filtered is placed in the vessel *e*, which is connected to *c*. The vessel is closed at its lower end by the conical stopper *f*, fastened to the rod *g*. This rod is surrounded by a hollow spindle *h*, to which two stirrers, *i* and *i'*, are attached. The cone *k* serves to distribute the liquid evenly over the filtering surface when *f* is lifted.



(*Reproduced with permission from Z. Ver. deut. Zucker-Ind., 83, 1078.*)

FIG. 309. Apparatus for determining filterability of sugar juices.

Three hundred milliliters of the juice to be tested is poured into vessel *e*, the stopper *f* being kept firmly in the closed position. Then the hollow spindle is given three turns to mix the juice thoroughly and to prevent the precipitate from settling out. The stopper is now slowly lifted by the handle on top, and at the same moment a stop watch is started. The filtrate is collected in a measuring cylinder, and the time is noted during which 25, 50, 100, and 150 ml. of filtrate are obtained. The time elapsed is a measure of the filterability.

All the parts of the apparatus, including the filter paper, must be perfectly dry, otherwise the filter is liable to break when it is struck by the liquid. The juices must always have the same temperature,

[84] *Z. Ver. deut. Zucker-Ind.*, **83**, 1077 (1933); the apparatus is manufactured by Von Dolffs & Helle, Braunschweig, Germany.

around 85 or 90° C., when they are poured into the vessel; under this condition the heat losses during the operations are constant. The results are sufficiently reproducible for practical purposes, and check well with those obtained in the factory, no matter what type of filter is used there. If desired, the color of the filtrates may also be determined.

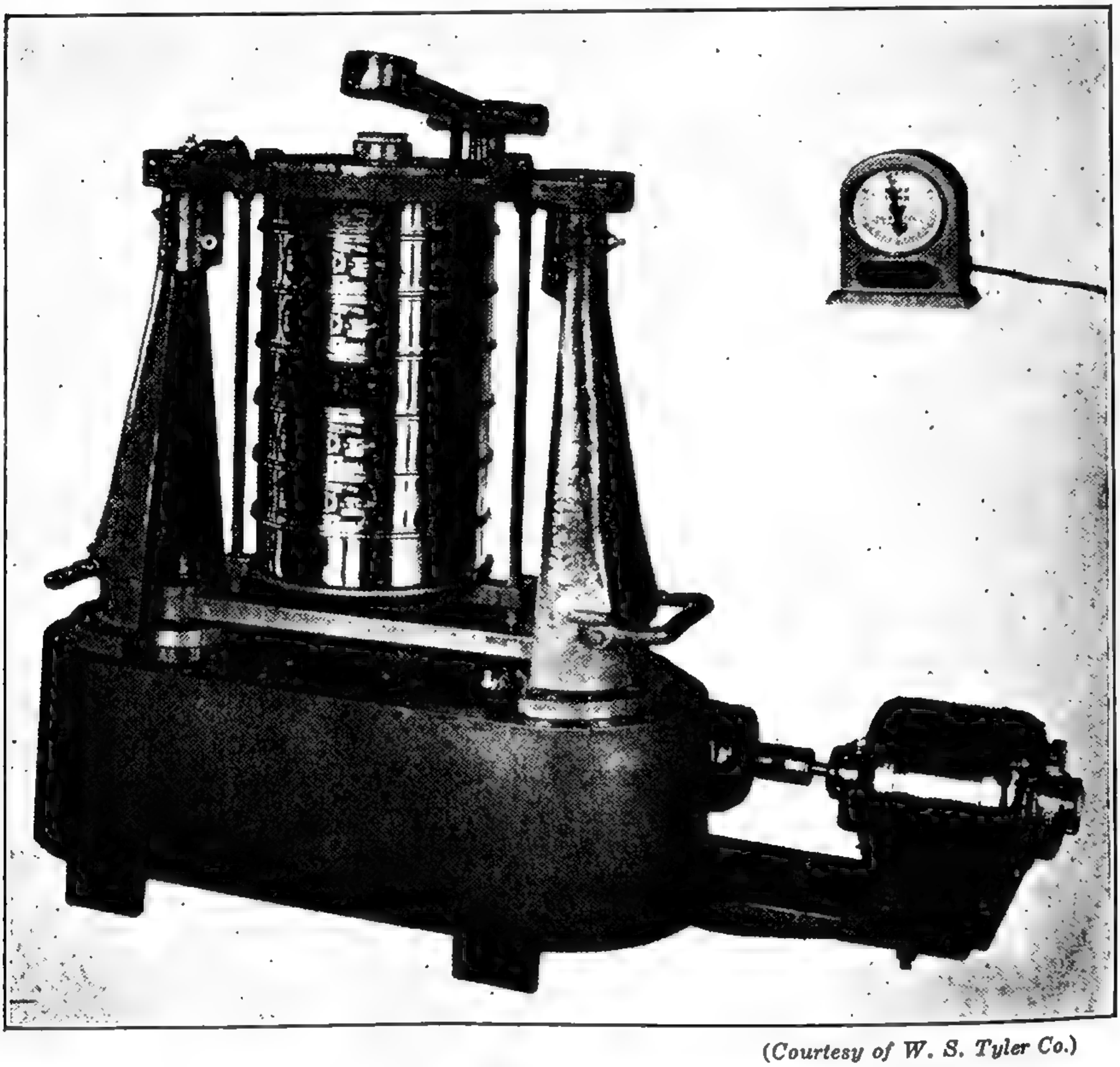
Grain Size of Raw and Refined Sugars. The size and uniformity of the crystals is an important factor in both raw and refined sugars. Large crystals purge more freely and are more easily washed than small ones. Fine grain mixed with larger crystals is very objectionable, because the small crystals fill the interstices between the larger ones during the purging and washing, and slow up the process; more wash water is required, and the finer crystals are partly dissolved.

Commercial grades of hard refined sugars are designated and sold in the United States according to the predominating size of grain, as coarse, medium, standard, fine, extra fine, coarse powdered, standard powdered, XXXX powdered, etc. The distribution of grain size in refined sugars is easier to determine than that in raw sugars, and the method used is therefore described first.

Determination of Grain Size by Dry Sifting. Standard sieves and a standard shaking machine are used for this purpose. In the United States, the sieves and the Ro-Tap shaker, Fig. 310, manufactured by the W. S. Tyler Co., are generally employed. Depending on the grade of the sugar, any three or more of the following sieve sizes are in use: Tyler No. 4, 6, 8, 10, 14, 20, 28, 30, 35, 40, 48, 50, 60, 65, 80, 100, 150, and 200. The sieves selected are assembled into a nest, with the coarsest sieve on top, and a receptacle for the sugar passing through the finest sieve at the bottom. In the case of granulated sugar, 100 g. is transferred to the top sieve, the cover is put on, the set is placed in the Ro-Tap shaker, and shaking is continued for a definite time, usually 5 or 10 minutes. Each fraction is then brushed into a weighing dish, weighed separately, and reported as, e.g., "on 4-mesh," etc., with the exception of the last which is designated as, e.g., "through 60-mesh." The grams of each fraction give the percentage directly.

With powdered sugars a smaller quantity is generally used for the analysis, 20 g. being sufficient. After shaking for a definite time, the top sieve is removed and put on a piece of glazed paper, and the sugar is brushed on the screen with a soft brush until no more passes through. The siftings are added to the sugar in the next sieve, and the process is repeated on to the lowest sieve. Each fraction is then weighed as described for granulated sugars, and the results in grams are multiplied by 5 to convert them into percentages.

Meade[85] has developed a simple graphic method which gives at a glance an approximate idea of the grain size and uniformity of a granulated sugar. The grain character was found to be directly proportional to the percentage remaining on the 30-mesh sieve, and indirectly proportional to that passing through the 50-mesh sieve. In the diagram, Fig. 311, the former is plotted upward and the latter downward, and the two points found are connected by a straight line. A sugar giving a hori-



(*Courtesy of W. S. Tyler Co.*)

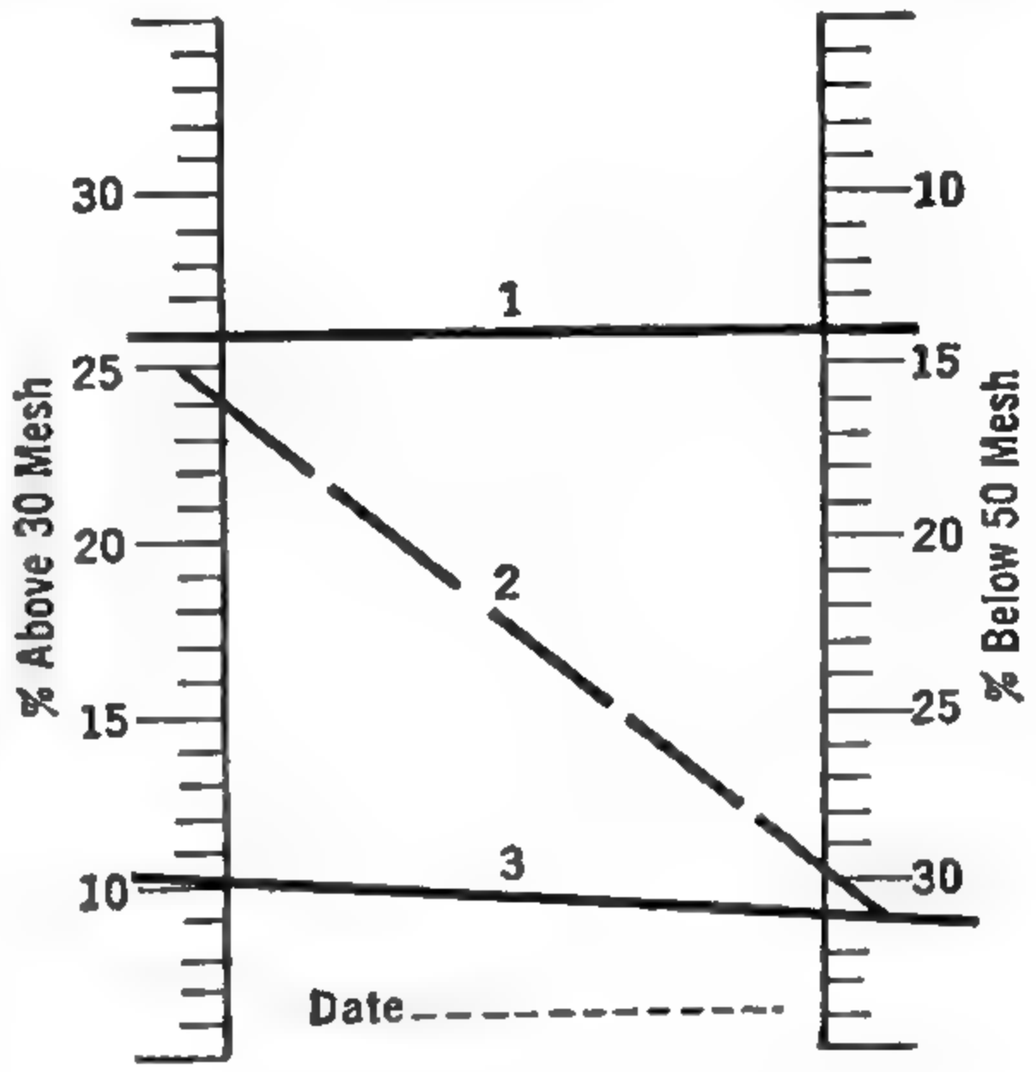
FIG. 310. Ro-tap testing sieve shaker.

zontal or slightly inclined line, as Nos. 1 and 3, is of acceptable, regular grain, No. 1 being rather coarse, No. 3 very fine. A composition like that shown by No. 2 indicates an irregular grain, consisting of large proportions of both coarse and fine.

[85] "Spencer's Handbook for Cane Sugar Manufacturers," 7th ed. by Meade, p. 188, 1929.

Dry Sifting of Raw Sugars. Raw sugars in their natural state are usually too sticky to be sifted. However, Harman[86] has reported that they may be readily prepared for sifting, by drying in a rotary drier holding about 4 pounds of sugar. In a humid atmosphere such dried sugars soon attract moisture again, and it is therefore usually preferred first to remove the molasses film. In the method used at the Java Sugar Experiment Station[87] the sugar is vigorously shaken for half an hour with sugar-saturated, 90 per cent alcohol. The alcohol is removed by placing the moist sugar in a twill bag, and spinning in a centrifuge. The sugar is dried in the sun, or in a current of warm air, and then sifted through Tyler standard sieves.

The Research Institute of the Czechoslovakian Sugar Industry[88] uses a slightly different method for raw beet sugars. About 250 to 300 ml. of 75 per cent alcohol, saturated with raw sugar, is added to 150 g. of the sample in a beaker, and the mixture is stirred every 5 minutes for half an hour. It is allowed to stand at rest for another half hour, and the alcohol is decanted, if necessary through filter paper. The remainder is centrifuged in a Collatz centrifuge (see p. 1043). Then about 50 ml. of 96 per cent alcohol, saturated with refined sugar, is added in the centrifuge and mixed with the sugar for 5 minutes, and the mixture is again centrifuged. The sugar is dried for 2 hours in the air or for 15 minutes in a drying oven, weighed, and sifted through standard sieves. The weight of the fines retained on the filter paper and of the sugar passed during the first centrifuging is added to that of the sugar passing through the smallest sieve used.



(*Courtesy of Mr. G. P. Meade.*)

FIG. 311. Chart showing result of sieve test.

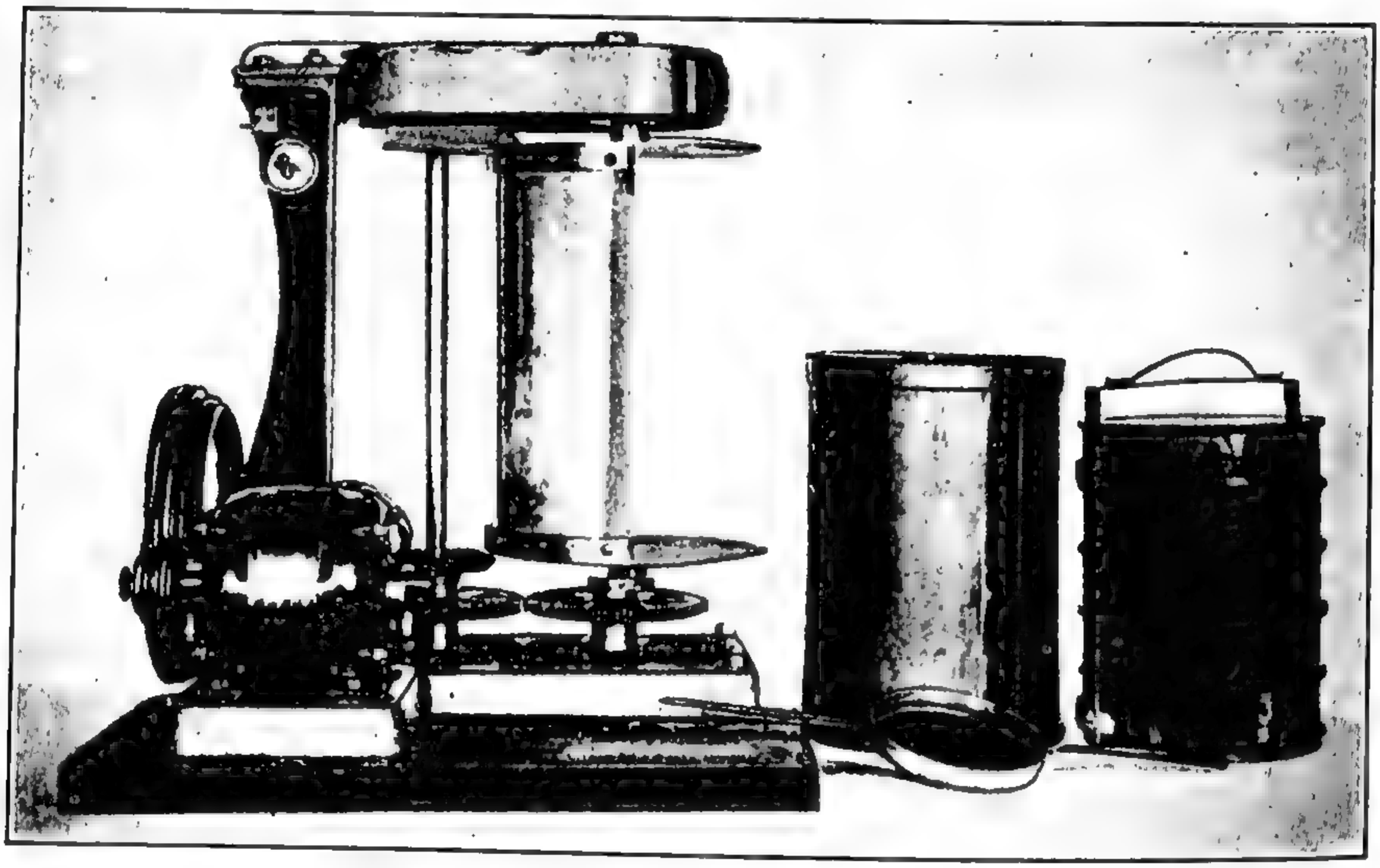
Wet Sifting Method of the Hawaiian Sugar Planters' Experiment Station. This is a modification of a procedure originally devised by Welle, and is carried out as follows:

[86] *Proceedings 5th Congress Intern. Soc. Sugar Cane Technologists*, p. 765, 1935.
[87] Prinsen Geerligs, *Intern. Sugar J.*, 34, 393 (1932).
[88] Šandera and Mirčev, *Z. Zuckerind. čechoslovak. Rep.*, 61, 217 (1936/37).

Apparatus. The apparatus used for the preliminary preparation of the sample is a Coles Shaker, a simple machine giving an up-and-down movement, and formerly much used for preparing milk drinks.

The sieve shaker used in separating the grains (Fig. 312) consists of a brass cylinder, which contains the alcohol solution in which the sieves are immersed, belt-driven from a motor and back-geared to produce a rotary motion at the rate of 100 revolutions per minute.

A small hand-driven centrifugal machine is used for throwing off the excess of alcohol solution clinging to the sieves and sugar, before they are dried. For drying, a tall gas oven is used, in which the sieves are placed in the reverse order to that in the shaking machine.



(Reproduced with permission from Expt. Sta., Hawaiian Sugar Planters' Assoc.)

FIG. 312. Apparatus for wet sifting of raw sugars.

The sieves used for separating the sugar are of 14-, 20-, 28-, 35-, and 100-mesh Tyler standard screen, 8 inches in diameter.

A small sieve of 200-mesh screen, 6 inches in diameter, with 1-inch rim and a short handle (Fig. 312), is used for receiving the sample after the preliminary shaking.

Alcohol Solutions Used. The solution (No. 1) in the cylinder of the sieve shaker is ordinary denatured alcohol, 90 per cent, saturated with white sugar and lime.

For removing the molasses from the sugar a solution (No. 2) is made up as follows: 105 g. of white sugar is dissolved in 100 ml. distilled water and 900 ml. denatured alcohol (90 per cent) added and mixed. After standing overnight,

to allow the excess of sugar to crystallize out, it is ready for use. At 28° C. it has a specific gravity of 0.90.

Method of Separation. Fifty grams of the sugar is weighed out, placed in one of the glasses of the Coles Shaker, covered with the No. 2 alcohol solution, allowed to stand half an hour, and shaken with about ten rapid turns of the machine. The sample is then transferred to the small sieve, which rests on a large funnel, using No. 1 alcohol solution in a wash bottle, to free the grains from the molasses solution, and from there to the top of the nest of sieves arranged in the order 14, 20, 28, 35, 100 downward. With a tight-fitting cover on the sieves they are placed in the brass cylinder, containing enough No. 1 alcohol solution to cover them, and shaken for 15 minutes. After the separation the sieves are drained in the centrifugal machine, a small sample is taken from each for examination with the microscope, and the sieves and sugar are dried in the oven for 20 minutes at 120° C. After cooling, they are weighed, and the net weight of sugar of different-sized grains is found by subtracting the weight of the empty sieves after they have been immersed in the alcohol solution and dried. The per cent of different-sized grains is calculated from their total weight.

Grain-Size Determination by Magnification. Another method for ascertaining the grain size of raw and refined sugars is based on magnification and measurement by means of a ruled field. With this method it is not possible to determine the weight percentage of the various fractions, but merely the relative number of the different sizes of grain. It has the advantage, however, that conglomerate grain and the presence of impurities occluded in the crystals can be readily detected. The two methods thus supplement each other. Any microscope giving suitable magnification may be used; the binocular type is preferable because it yields a stereoscopic view of the crystals. The sugar is gently rubbed with glycerol or sugar-saturated alcohol and evenly distributed on a stage micrometer disk ruled in squares of known dimensions. The proportion of the various sizes can be readily estimated.

Meade's Projectoscope.[89] With this apparatus the magnified image of the crystals is thrown on a screen. An improved form, Fig. 313, is described by Meade as follows:[90]

It consists of a Bausch and Lomb Micro-Tessar lens, 72 mm. focus (*C*), mounted in a rack-and-pinion combination (*D*) with suitable condenser (*F*). The light source is a 6-volt, 108-watt Mazda lamp in housing (*G*), the whole being mounted on an optical bed (*H*) with a 45° mirror at the top to direct the projected image horizontally. The screen, preferably a smooth plaque of

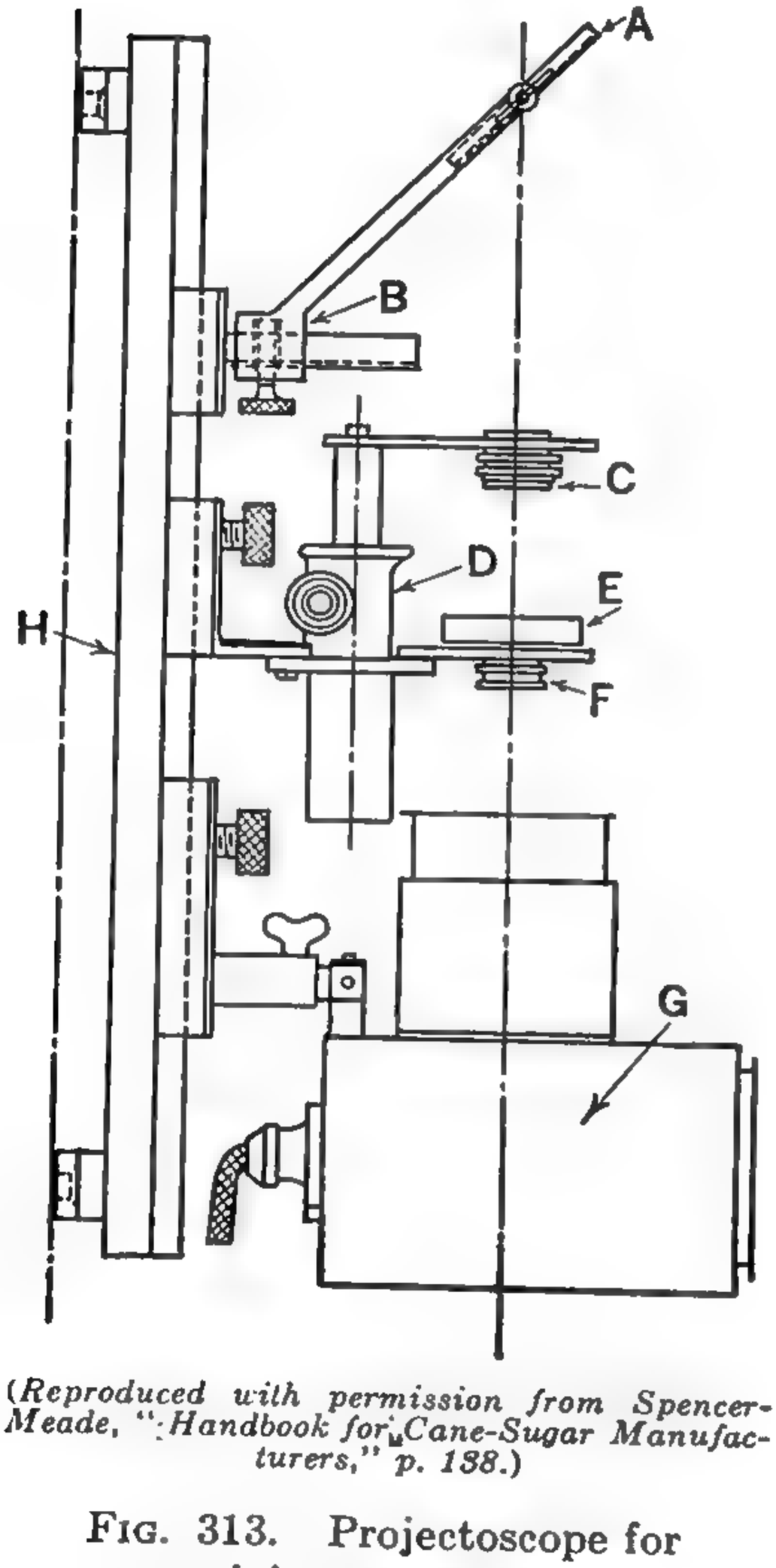
[89] *Ind. Eng. Chem.*, 13, 712 (1921).

[90] "Spencer's Handbook for Cane Sugar Manufacturers," 7th ed. by Meade, p. 138, 1929.

plaster of Paris about 18 inches square by 0.5 inch thick, is placed 73 cm. away to give a magnification of 10 diameters.

A small amount of the sugar to be examined is placed in a Petri dish (*E*), covered with sugar-saturated alcohol, the grains being separated by rubbing gently with the ball of the finger.

The surface of the plaque is ruled in squares of such dimensions that the approximate proportion of the various-sized crystals can be readily estimated. Meade uses a scale in steps of 0.2 mm. square, actual size (0.4, 0.6, 0.8 mm. square, etc.). The scale adopted by the Hawaiian Sugar Planters' Association is as follows: standard, 1 mm. square; very small, less than 0.5 mm. square; small, 0.5 to 0.75 mm.; medium, 0.75 to 1 mm.; large, 1 to 1.5 mm.; very large, over 1.5 mm. square.



(*Reproduced with permission from Spencer-Meade, "Handbook for Cane-Sugar Manufacturers," p. 138.*)

FIG. 313. Projectoscope for examining sugar crystals.

Other microprojectors have been described by Alewijn,[91] Stare,[92] Gollnow[93] and Šandera and Mirčev.[94] Some of these may be used also for the preparation of photomicrographs, to obtain a permanent record.

Average Grain Size. Although the grain-size distribution, determined by a sieve analysis, is a satisfactory specification for many purposes, it is frequently desirable to express the results obtained in a single figure. The average grain size is usually calculated in the following manner. The weight percentage of each grain fraction is multiplied by the average of the sieve openings for the same fraction, and the sum is divided by 100. The calculation is illustrated by the following example:

[91] *Arch. Suikerind.*, **38**, 557 (1930).
[92] *Deut. Zuckerind.*, **57**, 401 (1932).
[93] *Deut. Zuckerind.*, **58**, 761 (1933).
[94] *Z. Zuckerind. čechoslovak. Rep.*, **58**, 321 (1933/34).

Fraction	Average Sieve Opening, mm.		Weight of Fraction, per cent	
On 1.65 mm.			0	= 0.00
Through 1.65 mm., on 1.17 mm.	1.42	×	4	= 5.68
Through 1.17 mm., on 0.83 mm.	1.00	×	29	= 29.00
Through 0.83 mm., on 0.59 mm.	0.71	×	49	= 34.79
Through 0.59 mm., on 0.30 mm.	0.445	×	14	= 6.23
Through 0.30 mm.	0.15	×	4	= 0.60
				76.30

The average grain size is 76.30 ÷ 100 = 0.76 mm.

The common types of raw and refined sugar usually pass completely through the 1.65-mm. sieve, unless they contain crystal conglomerates, which are reported separately. If single crystals larger than 1.65 mm. are present their average size is estimated for the calculation of the average grain size.

Specific Grain Size. According to Douwes Dekker[95] the result of the method just described is misleading because in each fraction the smaller particles preponderate over the larger ones. More exact results are obtained by first determining the specific surface of each fraction and then converting this into the specific grain size. The specific surface is defined as the ratio of the total surface of all the crystals to the total surface of an equal weight of crystals of the same substance having a diameter of 1 cm.

The specific surface U of a large number of spherical particles differing in size may be calculated by the formula of Zunker:

$$U = \frac{4.343}{\log d_2 - \log d_1}\left(\frac{1}{d_1} - \frac{1}{d_2}\right)$$

where d_1 and d_2 are the diameters of the smallest and largest particles, respectively. The formula presupposes that equal weights of all the different grain sizes between d_1 and d_2 are present. For equal weights of material the total surface is inversely proportional to the diameter of the particles, and it follows that, if the diameter d_2 of the largest particles equals 1 cm., the specific surface of the smallest particles, U_1, equals $1 \div d_1$; consequently the diameter of the smallest particles equals $1 \div U_1$, or, if the diameter is expressed in millimeters, it equals $10 \div U_1$. This figure is termed the specific grain size.

Applying the same reasoning to a number of fractions of varying size but equal weight, the specific surface of the entire sample can be derived by multiplying the weight percentage of each fraction by the

[95] Private communication.

corresponding U value of that fraction, calculated by the formula of Zunder, adding all the products together, and dividing the sum by 100. The specific grain size, in millimeters, is then found by dividing the specific surface into 10. The integration process just outlined must not be applied to the specific grain size of each fraction, but to its specific surface.

The formula of Zunker may be applied without serious error to non-spherical particles as long as they are of the same shape, which is approximately true for the usual sugar crystals.

The specific surface of sugar crystals of varying size has been calculated at the Java Sugar Experiment Station for the fractions obtained by the use of Tyler sieves Nos. 10, 14, 20, 28, and 48, with the following results, in round figures: first fraction (on 1.65 mm.), 4.8; second fraction (through 1.65, on 1.17 mm.), 7.2; third fraction (through 1.17, on 0.83 mm.), 10.0; fourth fraction (through 0.83, on 0.59 mm.), 14.3; fifth fraction (through 0.59, on 0.30 mm.), 25.0; sixth fraction (through 0.30 mm.), 50.0.

Using again the sieve analysis given on p. 1063 as an example, the specific surface of the sugar sample is found as follows:

First fraction	$0 \times 4.8 =$	0.0
Second fraction	$4 \times 7.2 =$	28.8
Third fraction	$29 \times 10.0 =$	290.0
Fourth fraction	$49 \times 14.3 =$	700.7
Fifth fraction	$14 \times 25.0 =$	350.0
Sixth fraction	$4 \times 50.0 =$	200.0
		1569.5

The specific surface is then $1569.5 \div 100 = 15.695$, and the specific grain size is $10 \div 15.695 = 0.64$ mm. This is 0.12 mm. less than the average grain size, 0.76 mm.

Weight per Unit Volume of Granulated Sugars. An approximate idea of the average grain size of granulated sugars may also be gained by the following method used in several American refineries. An Erlenmeyer flask of about 1-liter capacity is filled level full with distilled water at 20° C., weighed, and the weight of the empty flask is deducted to obtain the weight of the water. An 8-inch funnel, the stem of which has been cut off, leaving an outlet at least ¼ inch in diameter, is suspended centrally above the empty, dry flask so that the lower end of the funnel is 1 inch above the rim of the flask. A felt pad is placed under the flask to prevent jarring. The outlet of the funnel is closed with the thumb, and the funnel is filled with slightly more of the sugar than the flask will hold. The thumb is removed, the flask filled to overflowing with the sugar, and the latter leveled off. The outlet of the

funnel is closed again, the sugar transferred from the flask to the funnel and run into the flask a second time. The sugar should now fill the flask level full. If it does not, a little sugar is removed or added, until in repeated tests the sugar just fills the flask. The total weight is determined, and the weight of the sugar divided by the weight of the water. The resulting figure varies inversely with the average grain size of the sugar.

II. Determination of Individual Non-Sugars or Groups of Non-Sugars

While a knowledge of the proximate composition of sugar products (sucrose, reducing sugars, moisture, ash, organic non-sugars) is sufficient for many purposes, the chemist is frequently called upon to determine individual non-sugars or groups of non-sugars, both inorganic and organic, and for this reason a few selected methods that may be used for this purpose are described here.

The quantities of potash, soda, lime, magnesia, other fixed bases, and of silica may usually be ascertained by an analysis of the carbonated ash according to the usual methods. But the chlorine, sulfur, and phosphorus are partially lost during the incineration, and they must be determined in the product itself without previous ashing.

Determination of Sulfates. The Hawaiian Sugar Technologists' Association uses the following procedure[96] for estimating sulfates in raw sugars. Twenty grams of the sugar is dissolved in distilled water; the solution is made up to a total volume of 200 ml. and filtered through kieselguhr which must be free from sulfates. One hundred milliliters of the filtrate is pipetted into a 400-ml. beaker, diluted with 100 ml. of water, and acidified with 5 ml. of 20 per cent hydrochloric acid. The solution is heated to boiling and precipitated with a slight excess of hot 1 per cent barium chloride solution. Boiling is continued for 3 minutes, and the solution is allowed to stand overnight. The barium sulfate is filtered off through a tared Gooch crucible, washed with hot water, dried, ignited, cooled in a desiccator, and weighed. The weight in grams, multiplied by 3.43, gives the percentage of sulfur trioxide in the sugar. The same method may also be applied to other sugar products, by varying the amount used for the analysis according to the approximate content of sulfur trioxide.

A rapid colorimetric micromethod for determining sulfates in cane juices has been described by Chu and Hance.[97] The excess barium

[96] "*Chemical Control for Cane Sugar Factories*," p. 53, 1931.

[97] *Hawaiian Planters' Record*, 43, 137 (1939).

chloride is converted into a red-colored compound by the addition of sodium rhodizonate, and the solution is compared with permanent standards prepared from sodium dichromate.

Sulfates in White Sugars. Ambler, Snider, and Byall[98] give the following directions for this determination. One hundred grams of the sugar is dissolved in 100 ml. of water, the solution is acidified with 5 ml. of concentrated hydrochloric acid, and 10 ml. of a 10 per cent barium chloride solution is added at room temperature. The mixture is thoroughly stirred and allowed to stand overnight. The barium sulfate is filtered off on a Gooch crucible with a thick asbestos mat, washed with hot water, dried, heated in a muffle to 550° C., cooled, and weighed. Experience shows that it is better not to heat the solution with the barium precipitate because it becomes discolored and turbid, through the decomposition of reducing sugars.

If the sulfate content of the sugar is very low it is preferable to dissolve 100 g. in 200 ml. of a stock solution containing about 0.01 per cent of sulfuric acid and 50 ml. of concentrated hydrochloric acid per liter. The sulfuric acid contained in 200 ml. of the stock solution is determined by precipitation with barium chloride, and the result is deducted from that found upon the sample.[99]

Determination of Sulfur Dioxide. Several countries impose legal restrictions on the amount of free and combined sulfur dioxide that may be present in food products, such as sugars and molasses made by the sulfitation process. The sulfur dioxide may be estimated by distillation in the presence of a mineral acid and determination in the distillate, by direct titration, or by a differential gravimetric method.[100]

Distillation Method of Monier-Williams for Total Sulfur Dioxide. This has been adopted as the official method of the Association of Official Agricultural Chemists. The procedure, as described in the Association's Methods of Analysis,[101] is as follows:

Connect a 750-ml. round-bottomed Pyrex flask (*B*) (Fig. 314) to a sloping reflux condenser (*D*), the lower end of which is cut off at an angle. (Monier-Williams prefers using an upright round-bottomed flask with two necks.) Pass carbon dioxide from a generator through a sodium carbonate solution in *A* to remove chlorine. Also connect a dropping funnel (*K*) to *B* by the three-holed stopper *C*. Use the tube *E* to connect the upper end of the condenser

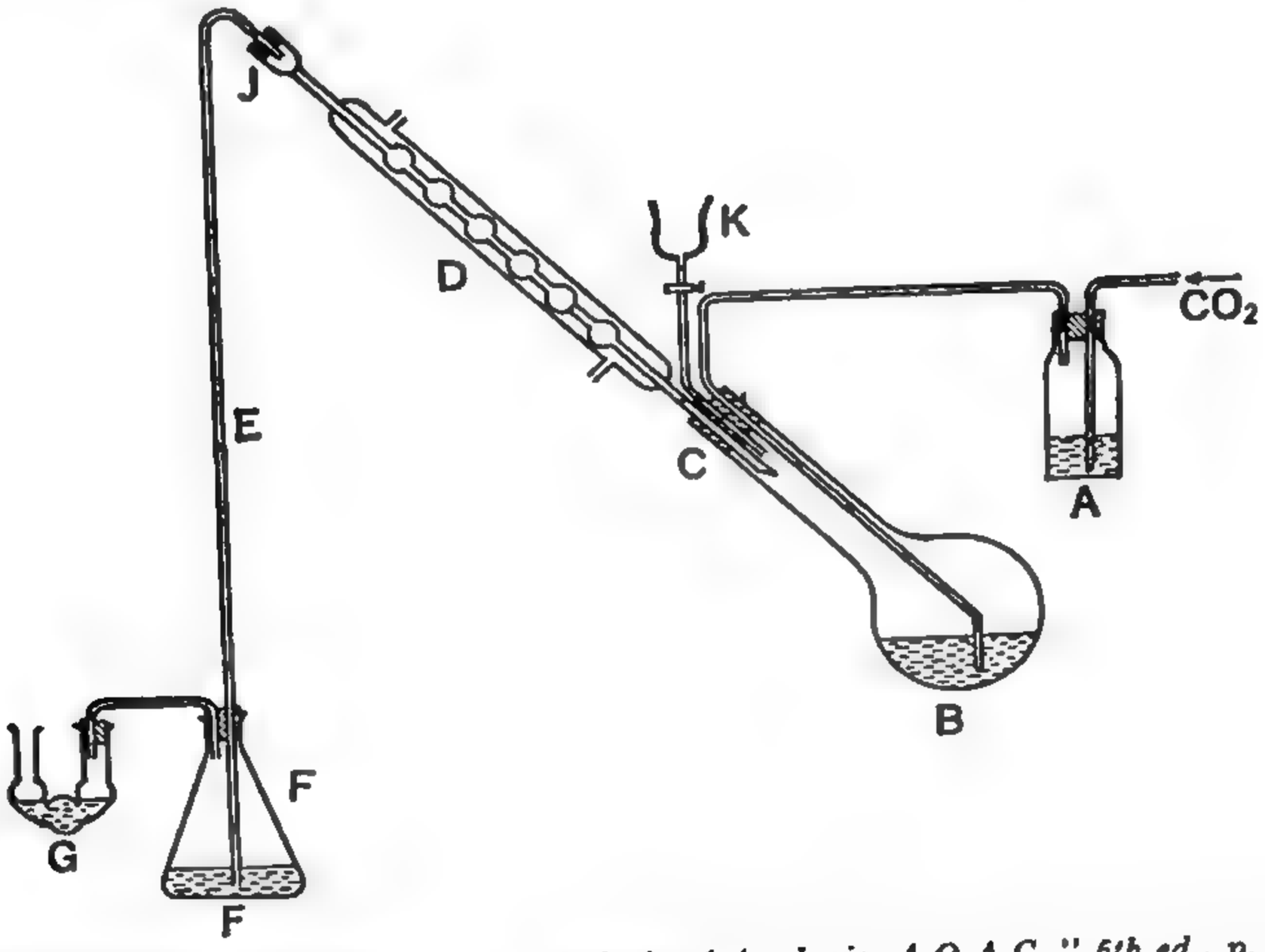
[98] *Ind. Eng. Chem., Anal. Ed.*, 3, 339 (1931).

[99] "Methods of Determining the Uniformity of Quality of White Sugars," U. S. Dept. of Agriculture, Carbohydrate Research Division, May, 1940, p. 13.

[100] For the sulfide stain method see Ogilvie, *Intern. Sugar J.*, 28, 644 (1926); Spengler and Brendel, *Z. Ver. deut. Zucker-Ind.*, 77, 167 (1927).

[101] "Methods of Analysis, A.O.A.C.," 5th ed., pp. 463–465, 1940.

to a 200-ml. Erlenmeyer flask (*F*), which is followed by a Peligot tube (*G*). This delivery tube (*E*) extends to the bottom of the receiver. One Peligot tube has been found to be sufficient to catch traces of sulfurous acid swept through the flask *F*. Use rubber stoppers throughout. The receiver *F* contains 15 ml. of pure neutral 3 per cent hydrogen peroxide, while the Peligot tube contains 5 ml. Hydrogen peroxide usually contains free sulfuric acid. Start with 30 per cent hydrogen peroxide, dilute somewhat, and neutralize with barium hydroxide solution, using bromphenol blue solution as indicator. After the reagent has settled in the cold, filter from the barium sulfate, determine its strength by permanganate titration, and finally adjust to a 3 per cent strength. The bromphenol blue indicator in the hydrogen peroxide remains unaffected for some time.



(Reproduced with permission from "Methods of Analysis, A.O.A.C.," 6th ed., p. 464.)

FIG. 314. Monier-Williams apparatus for determination of sulfurous acid.

After connecting the apparatus, introduce into the flask 300 ml. of distilled water and 20 ml. of hydrochloric acid and boil for a short time in a current of carbon dioxide. Then add the food to be tested, adapting the procedure to the sort of food. Add liquids directly by means of the dropping funnel. In the case of easily transferable solids, first cool the contents of the flask somewhat, taking care to regulate the flow of carbon dioxide to avoid having the hydrogen peroxide drawn up in the delivery tube *E*. Then quickly introduce the food by removing the stopper *C*. With semi-solid foods, requiring more time to introduce into the flask, cool the flask contents by gradual immersion in cold water, and wash the food in quickly with recently boiled distilled water. After introducing the food, boil the mixture for 1 hour ($1\frac{1}{2}$ hours in the case of dried fruits) in a slow current of carbon dioxide, stopping the flow of water in the condenser just before the end of the distillation. This causes the con-

denser to become hot and drives over residual traces of sulfur dioxide retained in the condenser. When the delivery tube just above the receiver *E* becomes hot to the touch, remove stopper *J* immediately.

Wash the delivery tube and the Peligot tube contents into the flask *F*, and titrate the liquid at room temperature with 0.1 *N* sodium hydroxide, using bromphenol blue as indicator. The sodium hydroxide must be standardized with this indicator. Bromphenol blue is unaffected by carbon dioxide and also gives a distinct color change in cold hydrogen peroxide. One milliliter of 0.1 *N* sodium hydroxide = 3.2 mg. of sulfur dioxide, so that titration of small quantities of sulfur dioxide requiring less than 0.5 ml. of sodium hydroxide is not accurate. A gravimetric determination may be made after titration, the precipitation of barium sulfate being carried out at room temperature. After allowing the supernatant liquid to settle, filter, and wash the residual barium sulfate three times by decantation with boiling water. Determine a blank on the reagents, both by titration and gravimetrically, and correct the results accordingly.

Direct Volumetric Determination of Total Sulfur Dioxide.[102] From 50 to 100 g. of raw sugar, or a convenient quantity of molasses, etc., is dissolved in 200 ml. of water; 25 ml. of *N* sodium hydroxide solution is added and thoroughly mixed with the sugar solution. This breaks up the addition compounds of reducing sugars and sulfurous acid. A rapid stream of nitrogen is passed through the solution for at least 10 minutes, to expel the air. The solution is then acidified with 10 ml. of 35 per cent sulfuric acid, and immediately titrated with *N*/10 iodine solution and starch as indicator. A blank test is run with water instead of sugar solution, and the titer of the blank is deducted from the titer of the solution. One milliliter of the *N*/10 iodine solution is equivalent to 3.2 mg. sulfur dioxide. The results obtained by this method are usually higher than those of the distillation method, because sugar products contain other non-sugars which are oxidized by iodine.

Direct Volumetric Determination of Free Sulfur Dioxide. By "free" sulfur dioxide is meant that portion which is not combined with reducing sugars, but present in inorganic form. It is determined in the same manner as described above for the total sulfur dioxide, but the pretreatment with sodium hydroxide is omitted, and only 5 ml. of 35 per cent sulfuric acid is used.

The quantity of free sulfur dioxide varies with the dilution employed, because the addition compounds are partly hydrolyzed. In experiments by Zerban,[103] 25 ml. of undiluted sulfured cane juice absorbed 12.2 ml. *N*/10 iodine, but required 13.3 ml. when diluted with

[102] Ambler, Snider, and Byall, *Ind. Eng. Chem., Anal. Ed.*, **3, 339 (1931)**.
[103] *Louisiana Expt. Station Bull.* 103, p. 33, 1908.

100 ml. of water. When different samples are to be compared, it is therefore necessary to dilute all in the same way.

Correction for Iodine Absorbed by Other Non-Sugars. In the sugar factory it is usually possible to titrate with iodine a portion of the same material that has not been treated with sulfur dioxide, and to use the result as a blank.[104] The differential method of Haddon[105] may be used in all cases. Another solution of the product is prepared in the same way as for the titration of either total or free sulfur dioxide, but immediately before the titration an excess of hydrogen peroxide is added. This oxidizes the sulfur dioxide to sulfur trioxide, but does not appreciably affect the other non-sugars oxidizable by iodine. The difference between the titrations with and without hydrogen peroxide gives the sulfur dioxide.

Differential Gravimetric Method. In this method, due to Saillard,[106] the sulfates are determined in one portion of the sample, as shown on p. 1065. In another portion the sulfites are oxidized to sulfates by bromine, iodine, or hydrogen peroxide, the total sulfate is precipitated as barium sulfate, and the latter weighed. The difference between the weights of the two barium sulfate precipitates is converted into sulfur dioxide.

Sulfur Dioxide in White Sugars. According to Ambler, Snider, and Byall,[107] total and free sulfur dioxide in these sugars are best determined by the titration methods described above. One hundred grams of the sugar is dissolved in 125 to 150 ml. of water, and the titration is carried out with $N/100$ instead of $N/10$ iodine solution. Some sugars do not give the usual blue color of iodine-starch, but a fugitive violet. In such cases the violet color should persist for at least 2 minutes.

Determination of Organic Sulfur. Sugar products contain sulfur in the form of cystine and related cleavage compounds of proteids. A method for the determination of this form of sulfur in white sugars has been worked out by Ambler,[108] but it is rather laborious. The sulfur is split off by boiling with alkaline lead solution, the lead sulfide is decomposed with hydrochloric acid, and the hydrogen sulfide obtained is passed into a solution of dimethyl-*p*-phenylene diamine and ferric sulfate in sulfuric acid. The methylene blue formed is determined spectrophotometrically and expressed as cystine. For the details of the method the chemist is referred to the original article.

[104] Troje, *Deut. Zuckerind.*, 58, 625 (1933).
[105] *Rev. agr. Maurice*, No. 75 (May–June, 1934), p. 104.
[106] "Betterave et sucrerie de betterave," p. 104, 1913.
[107] *Ind. Eng. Chem., Anal. Ed.*, 3, 339 (1931).
[108] *Ind. Eng. Chem., Anal. Ed.*, 3, 341 (1931).

Determination of Chlorides. In white sugars or light-colored sirups and raw sugars the chlorine can be determined directly by Mohr's procedure, as shown by Ambler and Byall.[109] One hundred grams of the sugar is dissolved in 150 ml. of distilled water, and about 0.5 ml. of a saturated solution of potassium chromate is added. The solution is titrated with $N/50$ silver nitrate solution, until excess is indicated by the brown color of silver chromate.

When large quantities of coloring matter are present it is impossible to detect the end point. This difficulty may be overcome by oxidizing the coloring matter, as proposed by Davies.[110] Catenacci[111] has applied this procedure to the analysis of beet products. Ten grams of molasses, or an equivalent quantity of other product, is dissolved in 200 ml. boiling water in a 400-ml. beaker, 20 ml. of concentrated nitric acid is added, and an excess of $N/10$ silver nitrate solution is run in from a burette. When the solution has become lukewarm, a saturated solution of potassium permanganate is added in small portions at a time, the beaker being set in a pan with running water to cool it. When the solution has become pale yellow, 1 ml. of a saturated solution of ferric alum in 10 per cent nitric acid is added, and 5 ml. of ether is run on the top of the liquid. The excess silver nitrate is titrated back with $N/10$ potassium thiocyanate solution, until the ether layer shows a permanent pink color, due to ferric thiocyanate. The difference between the milliliters $N/10$ silver nitrate and the milliliters $N/10$ thiocyanate gives the milliliters of $N/10$ chlorine present in the product.

Method of Budlovský.[112] For exact determinations of chlorine in impure sugar products it is preferable to distil the hydrogen chloride by heating with sulfuric acid, and to determine the chlorine in the distillate.

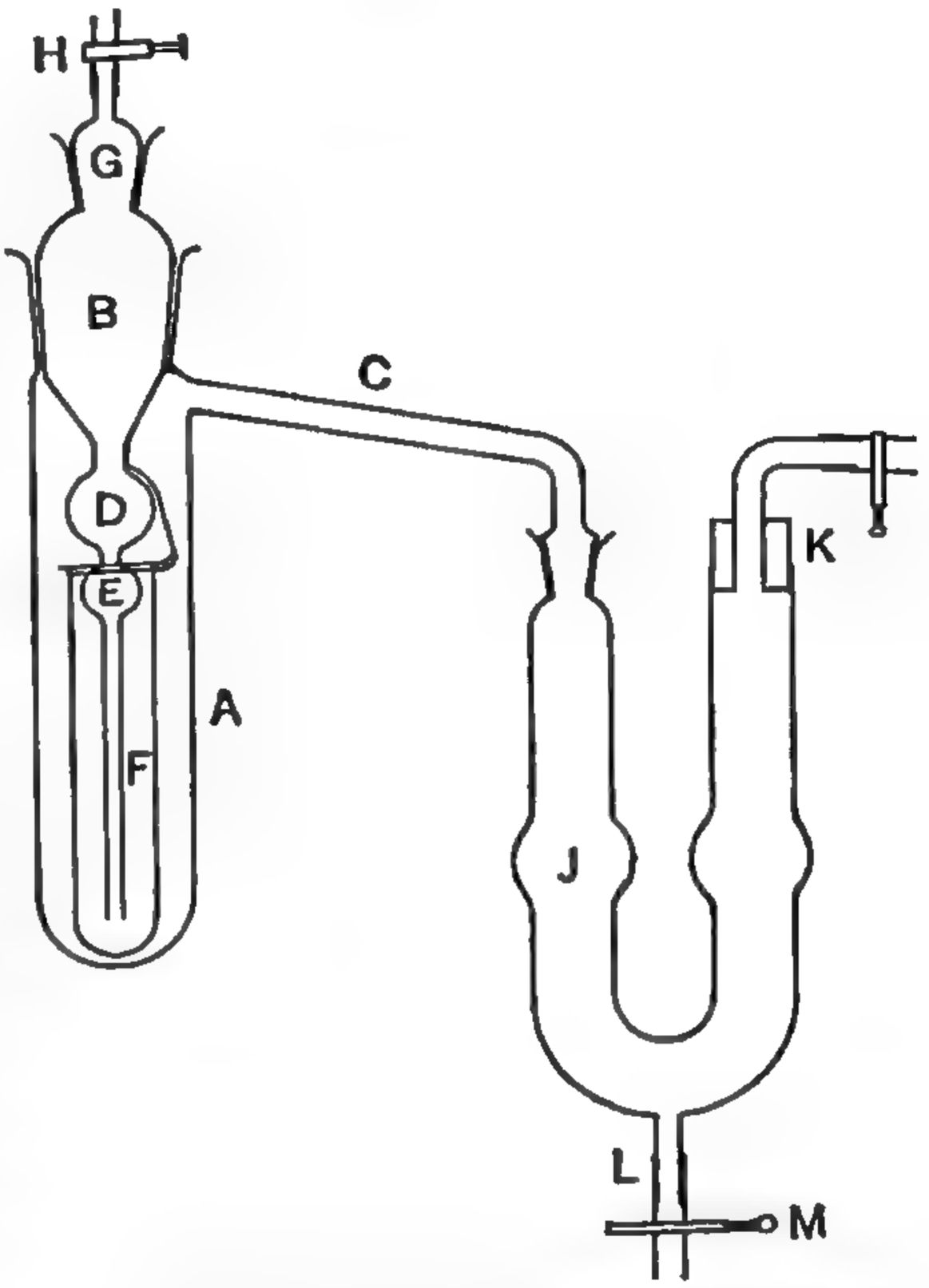
A weighed quantity of the product to be analyzed is placed in a test tube (*F* in Fig. 315), 13 cm. long and 2.5 cm. wide, dissolved in water, the solution acidified with nitric acid, and the chlorine precipitated in the usual manner with silver nitrate. The silver chloride remaining in colloidal dispersion is coagulated by producing a precipitate of ferric phosphate in the solution. Five milliliters or more of a solution containing 20 g. of ferric sulfate, 30 g. of crystallized disodium phosphate, and 10 g. of concentrated sulfuric acid in 1 liter is added, and then an equal volume of a solution of 40 g. sodium acetate, acidified with a few drops of glacial acetic acid, in 1 liter. Both these solutions must

[109] *Ind. Eng. Chem., Anal. Ed.*, **4**, 379 (1932).
[110] *Analyst*, **57**, 79 (1932).
[111] *Ind. saccar. ital.*, **26**, 458 (1933).
[112] *Z. Zuckerind. čechoslovak. Rep.*, **52**, 421 (1927/28).

be freed from chlorine during their preparation, by the addition of a few drops of silver nitrate solution before making up to the mark, allowing to stand for a few days, and filtering.

The test tube is spun in a centrifugal, and the supernatant liquid carefully decanted. The precipitate, without washing, is dried in the tube at 100° C., and a few small pieces of metallic copper are added to act as a catalyzer during the subsequent distillation.

The apparatus shown in Fig. 315 is constructed entirely of resistance glass, with ground-glass joints which are grooved around the edges. The seals are made tight by pouring a little concentrated sulfuric acid in the grooves. Only at the outlet *K* a rubber stopper is used. The mantle tube *A* is 24 cm. high by 5 cm. wide, the side tube *C* 12 cm. long and 1 cm. in diameter. The bulbs *D* and *E* are 3 and 2 cm. in diameter, respectively, and the U-tube *J* is 2.5 cm. wide. The outlets at *H*, *K*, and *M* end in short pieces of rubber tubing, with screw clamps.



(*Reproduced from Z. Zuckerind. Čechoslovak. Rep., 52, 424.*)

FIG. 315. Budlovský's apparatus for determination of chlorine.

Before the apparatus is assembled, tube *A* is filled with 90 ml. of a mixture of 4 parts by weight of concentrated sulfuric acid and 1 part of recently ignited sodium sulfate. The mixture is boiled in *A*, to remove all chlorine, a few pieces of gas carbon being added to prevent bumping. After thorough cooling, the tube *F*, with the silver chloride precipitate in it, is fastened to *D* with a platinum wire, and the stopper *B* is inserted in *A*. The U-tube *J* is filled with 60 ml. of distilled water, and connected with *C*. Stopcock *G* is opened, and 15 to 20 ml. of sulfuric acid, free from chlorine, is poured in through *B*, and the stopper is replaced. After all the seals have been tightened, a slow current of air, about 20 bubbles per minute, is drawn through the apparatus by means of an aspirator connected at *K*. Tube *A* is gradually heated with a free flame until the bath mixture begins to boil. Boiling is con-

Determination of Chlorides. In white sugars or light-colored sirups and raw sugars the chlorine can be determined directly by Mohr's procedure, as shown by Ambler and Byall.[109] One hundred grams of the sugar is dissolved in 150 ml. of distilled water, and about 0.5 ml. of a saturated solution of potassium chromate is added. The solution is titrated with $N/50$ silver nitrate solution, until excess is indicated by the brown color of silver chromate.

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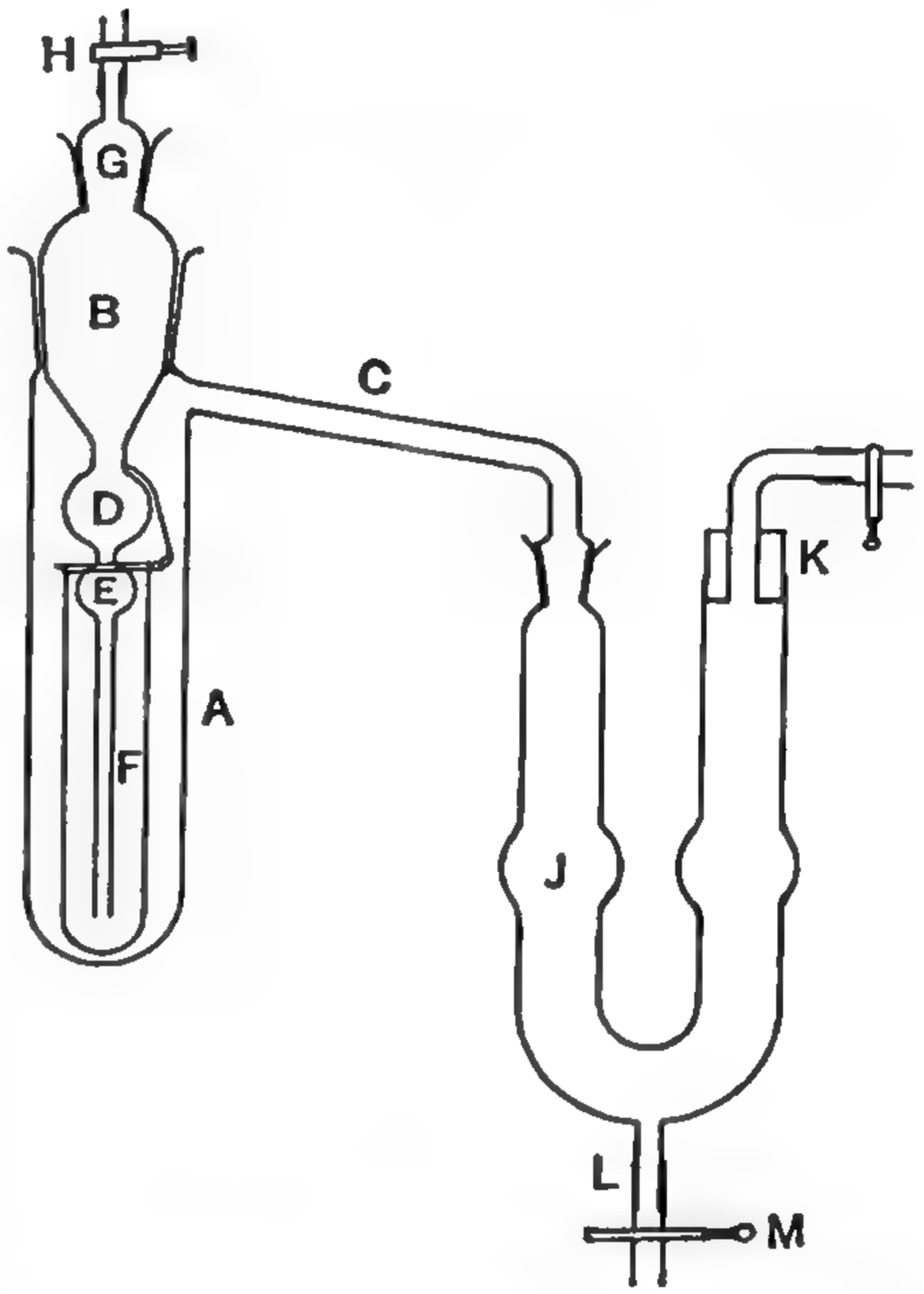
[112] *Z. Zuckerind. čechoslovak. Rep.*, **52**, 421 (1927/28).

be freed from chlorine during their preparation, by the addition of a few drops of silver nitrate solution before making up to the mark, allowing to stand for a few days, and filtering.

The test tube is spun in a centrifugal, and the supernatant liquid carefully decanted. The precipitate, without washing, is dried in the tube at 100° C., and a few small pieces of metallic copper are added to act as a catalyzer during the subsequent distillation.

The apparatus shown in Fig. 315 is constructed entirely of resistance glass, with ground-glass joints which are grooved around the edges. The seals are made tight by pouring a little concentrated sulfuric acid in the grooves. Only at the outlet *K* a rubber stopper is used. The mantle tube *A* is 24 cm. high by 5 cm. wide, the side tube *C* 12 cm. long and 1 cm. in diameter. The bulbs *D* and *E* are 3 and 2 cm. in diameter, respectively, and the U-tube *J* is 2.5 cm. wide. The outlets at *H*, *K*, and *M* end in short pieces of rubber tubing, with screw clamps.



(*Reproduced from Z. Zuckerind. Czechoslovak. Rep., 52, 424.*)

FIG. 315. Budlovský's apparatus for determination of chlorine.

Before the apparatus is assembled, tube *A* is filled with 90 ml. of a mixture of 4 parts by weight of concentrated sulfuric acid and 1 part of recently ignited sodium sulfate. The mixture is boiled in *A*, to remove all chlorine, a few pieces of gas carbon being added to prevent bumping. After thorough cooling, the tube *F*, with the silver chloride precipitate in it, is fastened to *D* with a platinum wire, and the stopper *B* is inserted in *A*. The U-tube *J* is filled with 60 ml. of distilled water, and connected with *C*. Stopcock *G* is opened, and 15 to 20 ml. of sulfuric acid, free from chlorine, is poured in through *B*, and the stopper is replaced. After all the seals have been tightened, a slow current of air, about 20 bubbles per minute, is drawn through the apparatus by means of an aspirator connected at *K*. Tube *A* is gradually heated with a free flame until the bath mixture begins to boil. Boiling is con-

tinued for 10 minutes, which is sufficient to drive the hydrogen chloride over into the receiver. If the reaction mixture should foam, the heating must be interrupted for a moment. Air is drawn through the apparatus for a while longer. Then the contents of the U-tube are washed through *L* into a beaker until about 200 ml. of liquid has been collected. The solution is boiled to remove the sulfur dioxide formed by reduction of the sulfuric acid, and the chlorine is determined in the distillate by any convenient method.

Determination of Silica and of Inorganic and Organic Phosphorus in White Sugar. The following method of Byall and Ambler[113] is based on the reduction of phosphomolybdic and silicomolybdic acid to molybdenum blue.

These reagents are used:

Molybdate Solution: Twenty-five grams of ammonium molybdate is dissolved in 300 ml. of water and to this is added 200 ml. of dilute sulfuric acid prepared from 75 ml. of the concentrated acid.

Hydroquinone Solution: One-half gram of hydroquinone is dissolved in 100 ml. of water to which a drop of sulfuric acid has been added to retard oxidation.

Sulfite Solution: Twenty grams of sodium sulfite is dissolved in 100 ml. of water. The solution should be freshly prepared.

Standard Phosphate Solution: A solution of 0.4394 g. potassium dihydrogen phosphate in 1 liter total volume is prepared; 25 ml. of this solution is then diluted to 200 ml. This gives a solution which contains 0.0287 mg. phosphorus pentoxide, equivalent to 0.0363 mg. silicon dioxide in 1 ml.

Determination of Total Silica and Phosphorus. A platinum dish is cleaned by fusing in it a mixture of equal parts of anhydrous sodium carbonate and potassium carbonate, followed by thorough washing. Five grams of the sugar is weighed into the dish and mixed with 0.1 g. each of the carbonates. The contents of the dish are carefully charred, and ignited in the muffle at 550° C. When the ash is perfectly white, it is fused over a free flame to a clear melt. The dish is cooled, and the cake dissolved with about 15 ml. of water to which 1 ml. of dilute acetic acid (1 volume glacial acid plus 4 volumes of water) has been added. The solution is transferred to a 100-ml. Nessler tube, 5 ml. of the molybdate solution and 1 ml. each of the sulfite and the hydroquinone solutions are added, and the volume is completed to 100 ml. with water. At the same time standards for colorimetric comparison are prepared by measuring quantities from 0.1 to 4 ml. of the standard phosphate solution into other Nessler cylinders, adding the reagents, and diluting with

[113] *Ind. Eng. Chem., Anal. Ed.*, **4**, 325 (1932).

water to 100 ml., as just described. After 30 minutes' standing the unknown is matched with the standards. If the solution prepared from the sugar shows a yellow tint, due to iron, the comparisons are made by viewing through a Wratten filter K-3, No. 9. The results are recorded as milliliters of standard phosphate solution which matches the unknown. A correction is applied for silica and phosphorus in the reagents by running a blank determination.

Determination of Total Phosphorus. Another 5-g. portion of the sample is ashed with carbonate mixture as in the previous determination, but the ash is not fused. After cooling, the mass is dissolved in a little water and acidified with concentrated nitric acid (sp. gr. 1.42). The solution is evaporated to dryness on the steam bath; the residue is again moistened with nitric acid and evaporated to dryness once more, to render the silica insoluble. The final residue is dissolved in water, the silica is filtered off, and the filtrate and washings are collected in a 100-ml. Nessler tube. The reagents are added, and the volume is completed in exactly the same manner as in the determination of silica plus phosphorus. The unknown is compared with the phosphate standards, and the result is again expressed as milliliters of this solution, correcting for the total phosphorus in the reagents on the basis of a blank test.

Determination of Inorganic Phosphorus. Ten grams of the sugar is dissolved in a little water in a Nessler tube; the molybdate, sulfite, and hydroquinone reagents are added; the color is developed, matched with the standard phosphate solution, and corrected for the inorganic phosphorus in the reagents; the results are recorded as before.

Calculation of the Results. (*a*) Silica. The corrected value obtained in the determination of total phosphorus is subtracted from that obtained in the determination of silica plus phosphorus, and the result is multiplied by 0.0363. The product is multiplied by 200, to convert it into parts per million. (*b*) Organic Phosphorus. One-half the corrected value obtained for the inorganic phosphorus is subtracted from the figure obtained for the total phosphorus, and the result is multiplied by 0.0287. The product, multiplied by 200, gives parts per million, expressed as phosphorus pentoxide. (*c*) Inorganic Phosphorus. The corrected value for inorganic phosphorus is multiplied by 0.0287, and again by 100 to obtain the parts per million, expressed as phosphorus pentoxide.

In the determination of silica plus phosphorus the quantity of acetic acid added must be strictly adhered to because the color developed by silica varies with the *p*H of the solution. If the color developed in any of the solutions of the unknown is darker than that of the 4-ml. stand-

ard, less sugar should be used, or the solution should be suitably diluted and an aliquot used for the comparisons. The limit of error of the method is about 0.1 to 0.2 ml. of the standard solution. The silica found in white sugars is always greatly in excess of the organic phosphorus, and usually there are only traces of inorganic phosphorus.

A method for the determination of silica, similar to that of Byall and Ambler but applicable to all sugar-factory products, has been described by Černý.[114]

Determination of Phosphate in Raw Sugars and Other Products of Lower Purity. This determination can be made without previous ashing of the product. The following method is used by the Association of Hawaiian Sugar Technologists for cane juices,[115] but it is also applicable to raw sugars, etc. A solution containing 35 g. of chemically pure uranium acetate and 50 ml. of glacial acetic acid in 1 liter is prepared and titrated against a standard phosphate solution made by dissolving 14.718 g. sodium ammonium phosphate ($NaNH_4HPO_4 \cdot 4H_2O$) in water in a liter volumetric flask, adding 5 ml. of sodium acetate solution (100 g. sodium acetate plus 50 ml. glacial acetic acid per liter), and making up to the mark. Twenty-five milliliters of the standard phosphate solution is diluted to 100 ml. with water, and the uranium acetate solution is added from a burette until a few drops of the liquid withdrawn with a small pipette give a brown coloration with a little powdered potassium ferrocyanide on a spot plate, indicating the formation of uranium ferrocyanide. The solution is then heated to between 90° and 100° C., and the titration continued until the end point appears again. Exactly 25 ml. of the uranium acetate solution should be required for the titration; if necessary the strength is correctly adjusted. One milliliter of the uranium solution is equivalent to 5 mg. phosphorus pentoxide.

For the determination, 100 g. of the raw sugar, or a convenient quantity of other products, is dissolved in water, 1 ml. of 10 per cent ammonia is added, and the solution acidified again with acetic acid. The titration with the uranium solution is carried out in exactly the same manner as the standardization. Each milliliter of uranium solution indicates 0.005 per cent phosphorus pentoxide, if 100 g. of sample has been used.

According to Staněk and Vondrák[116] the phosphoric acid can also be determined by first oxidizing all the organic matter with concentrated

[114] *Z. Zuckerind. čechoslovak. Rep.*, **59**, 273 (1934/35). See also Davies, Gomez and Boon, *Intern. Sugar J.*, **40**, 105 (1938).

[115] "Chemical Control for Cane Sugar Factories," p. 54, 1931.

[116] *Z. Zuckerind. čechoslovak. Rep.*, **46**, 227 (1921/22).

sulfuric and nitric acids. When the solution has become completely decolorized it is diluted with water, and the phosphoric acid is precipitated as phosphomolybdate and determined by the usual methods.

Rapid Determination of Lime Salts in Sugar Products with Soap Solution. The usual method for the determination of calcium by precipitation as oxalate gives excellent results but is time consuming.[117] In 1876 Pellet[118] proposed to estimate lime salts in sugar products by titration with soap solution, as had been done for many years in water analysis. Spengler and Brendel recommend the following procedure.[119]

The soap solution is prepared from a solution of 20 g. potassium hydroxide in 200 g. of alcohol, to which 100 g. of the best grade of olive oil is added. The mixture is heated on the water bath under reflux until a few drops of the reaction mixture give a clear solution in water. The soap solution is poured into 3 liters of water in a large dish, and calcium chloride solution is added until no more precipitate is formed. The calcium soap is thoroughly washed by decantation with water and strained through cloth. The surplus water is removed as much as possible by pressing. The moist calcium soap is then transferred to a mortar and thoroughly mixed with 40 g. of solid potassium carbonate. The mixture is extracted repeatedly with 96 per cent alcohol in a flask on a water bath under reflux. The extracts are combined and filtered. The solution is further diluted with 70 per cent alcohol (by volume) and standardized with a solution containing 4.358 g. crystallized barium chloride ($BaCl_2 \cdot 2H_2O$) per liter. Ten milliliters of the barium chloride solution is pipetted into a 300-ml. Erlenmeyer flask and diluted with distilled water to 100 ml. Three drops of ammonia is added, and the soap solution is run in, in small quantities at a time, from a burette. After each addition the flask is closed with a cork and well shaken. At the beginning the foam formed on shaking disappears again rapidly, then more slowly, and at the end point a layer of foam 1 to 2 cm. high is obtained which persists on standing for 30 seconds. The standard soap solution is diluted so that 10.1 ml. is required for 10 ml. of the barium chloride solution. Then 10 ml. of the soap solution is equivalent to 10 ml. barium chloride solution or 0.01 g. CaO, the excess 0.1 ml. being necessary for the formation of a persistent foam layer.

The quantity of sugar product taken for the analysis should require

[117] In a simple routine method for factory control, described by Staněk and Pavlas, the calcium oxalate is estimated turbidimetrically; *Z. Zuckerind. čechoslovak. Rep.*, **61**, 329 (1936/37).

[118] *J. fabr. sucre*, **1876**, No. 49.

[119] *Z. Ver. deut. Zucker-Ind.*, **78**, 175 (1928).

not more than 25 ml. soap solution, preferably around 10 ml. In the case of juices containing very small amounts of lime salts, 100 ml. is used for the titration. If the lime content is higher, smaller quantities are measured or weighed, and diluted always to 100 ml. with distilled water. Three drops of ammonia is added, and the titration is carried out in exactly the same manner as described for the standardization. A correction of 0.1 ml. is applied to the soap solution used. The milligrams of CaO corresponding to corrected milliliters of standard soap solution are given in Table CXXXV.

TABLE CXXXV

MILLIGRAMS OF CaO CORRESPONDING TO MILLILITERS OF STANDARD SOAP SOLUTION

Soap solution	CaO	Soap solution	CaO	Soap solution	CaO
ml.	mg.	ml.	mg.	ml.	mg.
0.5	0.25	9	8.9	18	19.2
1	0.7	10	10.0	19	20.4
2	1.7	11	11.1	20	21.6
3	2.7	12	12.3	21	22.8
4	3.7	13	13.5	22	24.0
5	4.7	14	14.7	23	25.1
6	5.7	15	15.9	24	26.3
7	6.8	16	17.0	25	27.5
8	7.8	17	18.1	..	....

The values in the table are interpolated to tenths of a milliliter. One hundred milliliters of the water used for dilution, after addition of 3 drops of ammonia, should not consume more than 2 to 4 drops of standard soap solution for persistent foam formation.

Determination of Iron. Although the amount of iron in sugar products is quite small, it has a very detrimental effect on their color, because it forms dark-colored compounds with the polyphenols usually present, as has been shown by Schneller[120] and by others. Traces of iron impart a grayish tinge to the sugars. The total iron in low-purity products is determined by ashing at a low temperature, dissolving in the least possible quantity of hydrochloric acid, oxidizing any ferrous iron present with nitric acid or potassium chlorate, and estimating the iron colorimetrically with thiocyanate or ferrocyanide, or by the sulfide method described below. With light-colored products ashing is not necessary, but the comparison may be made directly on aqueous solutions. Ferric iron is determined with thiocyanate or ferrocyanide, ferrous iron with ferricyanide. The standards are prepared with fer-

[120] *Louisiana Expt. Station, Bull.* 157, 1916.

rous ammonium sulfate or ferric ammonium sulfate dissolved in a solution of iron-free sucrose of the same concentration as the unknown. To compensate for the color in the solution of the product to be analyzed, a tube filled with the solution of the product being tested, but without addition of the test reagent, is placed behind the standard tube, and a tube filled with water behind the tube containing the unknown plus the test reagent. A block comparator (see p. 561) is used for this purpose.

The most sensitive procedure for the determination of total iron is the colorimetric sulfide method of Eastick, Ogilvie, and Lindfield.[121] Ten grams of crystallized ferrous sulfate ($FeSO_4 \cdot 7H_2O$) is dissolved in a small quantity of water in a 1-liter volumetric flask, a few drops of sulfuric acid are added, and the volume is completed with a 50 to 60 per cent solution of iron-free sucrose. The sucrose retards the oxidation of the ferrous iron. The stock solution is diluted immediately before a test in the ratio of 1 to 100; 1 ml. of the diluted solution contains 0.02 mg. of iron. For testing light-colored products, 3 to 10 g. or more, depending on the iron content, is dissolved in water in a Nessler cylinder, and made up to 100 ml. The standards are prepared by placing increasing measured quantities of the diluted iron sulfate solution in a series of other Nessler cylinders and also making up to the mark in each. Then 2 ml. of freshly prepared ammonium sulfide solution is added to each tube, and the solutions are well mixed. The ammonium sulfide is made by saturating ammonia with hydrogen sulfide and then mixing with an equal volume of ammonia. The tubes are allowed to stand for 10 minutes, and the comparisons made. During this time any ferric iron present is also converted into ferrous sulfide. If the color of the sugar product should interfere, the block comparator method described above may be resorted to. With very dark products, the iron must be determined in the ash. Copper and lead are usually present in such small amounts that they do not interfere with this test.

Determination of Total Nitrogen. The organic and ammoniacal nitrogen may be estimated by the usual Kjeldahl method,[122] or by the micromethod of Staněk.[123] If betaine is present, as in beet products, the digestion with sulfuric acid must be continued for about an hour after the solution becomes colorless. A rapid method, requiring only 25 to 35 minutes for the complete digestion and distillation, has been described by Lundin, Ellburg, and Riehm.[124] The digestion is carried

[121] *Intern. Sugar J.*, **14**, 428 (1912).
[122] "Methods of Analysis, A.O.A.C.," 5th ed., pp. 25–26, 1940.
[123] *Z. Zuckerind. čechoslovak. Rep.*, 45, 323, 335, 347 (1920/21).
[124] *Z. anal. Chem.*, **102**, 161 (1935).

out with a mixture of concentrated sulfuric and phosphoric acids, to which strong hydrogen peroxide is added. Metallic copper and mercuric sulfate are used as catalysts, and later during the digestion potassium sulfate is introduced. The digestion is complete in 12 to 16 minutes, even in the presence of betaine. After cooling, the liquid is made alkaline and distilled without a condenser. For the details of the method, which requires special equipment, the chemist is referred to the original article. Riehm[125] has obtained excellent results with this method in the analysis of all sorts of beet products.

If the total nitrogen, including that in the form of nitrates and nitrites, is to be determined, the digestion is carried out with a mixture of sulfuric and salicylic acids, by the methods adopted by the Association of Official Agricultural Chemists.[126]

Total Nitrogen in White Sugars. These sugars contain so little nitrogen that a special Kjeldahl method must be used. The following procedure is recommended by Ambler and Byall.[127] The reagents used consist of:

Ammonia-Free Water. This is prepared by boiling distilled water with magnesium oxide, powdered so finely that a water suspension of it will pass through a 150-mesh sieve. The distillate is tested from time to time with Nessler's reagent, and when it is found to be free from ammonia it is collected in an ammonia-free container. This and all the other analytical operations must be carried out in a room free from ammonia and its volatile compounds, or in closed systems.

Magnesia Water. A suspension of the finely powdered magnesia in distilled water is concentrated by boiling to two-thirds or one-half of its original volume, cooling the remainder, and allowing it to settle. The clear or faintly turbid supernatant liquid is used.

Copper Oxide. Powdered copper oxide is heated to a bright red heat to burn off all nitrogenous matter. It is cooled, and stored in a tightly stoppered bottle.

Potassium Hydroxide Solution. This contains 200 g. of pure potassium hydroxide (sticks) in 1 liter, being prepared with magnesia water.

Sodium Hydroxide Solution. A saturated solution of pure sodium hydroxide (sticks) in magnesia water.

Nessler's Reagent. Fifty grams of potassium iodide is dissolved in a little cold water, and a saturated solution of mercuric chloride is added until a permanent precipitate begins to form. Then 400 ml. of a 50 per

[125] *Z. Zuckerind čechoslovak. Rep.*, **60, 156 (1935/36)**.
[126] " Methods of Analysis, A.O.A.C.," 5th ed., p. **27, 1940**.
[127] *Ind. Eng. Chem., Anal. Ed.*, **4, 34 (1932)**.

cent potassium hydroxide solution is added, the volume is made up to 1 liter, and the precipitate allowed to settle. The clear solution is drawn off as required.

Standard Ammonium Chloride Solution. Exactly 1.9093 g. of pure ammonium chloride is dissolved to 1 liter with ammonia-free water; 20 ml. of this solution is again diluted to 1 liter with ammonia-free water. One milliliter of the final solution contains 0.01 mg. nitrogen as ammonia.

All rubber stoppers and tubing used must be boiled out thoroughly with dilute sodium hydroxide solution. Before each determination the ammonia stills must be freed from nitrogen compounds by distilling a magnesia suspension until no color develops when 2 ml. of Nessler's reagent is added to 100 ml. of distillate.

Method. Kjeldahl flasks of 800-ml. capacity are used for the digestions, and are first cleared of ammonia by concentrating 600 ml. of magnesia suspension to half its original volume. The remaining suspension is poured out, and the flask used without further washing. From 10 to 25 g. of sugar, according to its nitrogen content, is dissolved in magnesia water to a volume of 100 ml., and 10 ml. of the solution is pipetted into the flask. One gram of copper oxide is added from the tip of a spatula, then 25 ml. of concentrated pure sulfuric acid and, very cautiously, 25 ml. of the potassium hydroxide solution. The mixture is carefully heated with a free flame until frothing ceases, and then with a stronger flame until the carbon is completely oxidized, and the solution is of a clear green color. This requires about 2 hours. After the mixture has cooled it is diluted with 400 ml. of magnesia water and made alkaline with 50 ml. of the saturated sodium hydroxide solution. A teaspoonful of the powdered magnesium oxide is added at the same time. The flask is immediately connected with the still and 200 ml. of distillate is collected. To each 100 ml. of distillate 4 ml. of Nessler's reagent is added, and after 15 minutes the color developed is compared with the colors obtained similarly from known quantities of the standard ammonium chloride solution diluted with ammonia-free water to 100 ml.

In series analyses it is convenient to measure the standards spectrophotometrically and to establish a curve from which the concentration of the unknown may be read on the basis of its transmittancy.

A blank determination is run with water, and the nitrogen found is deducted from that found in the determination with sugar solution. The result is expressed as parts of nitrogen per million of sugar. It includes the nitrogen in the form of nitrates.

Determination of Protein Nitrogen. Vondrák[128] has shown that the method of Stutzer, usually employed on feeding stuffs for this determination, gives too high results with sugar products because the cupric hydroxide precipitates also peptones and amino acids. Precipitation with tannin is free from this objection. The protein nitrogen may be determined by dissolving a suitable quantity of the sugar product in water to a total volume of 100 or 200 ml., adding a slight excess of a 10 per cent tannin solution, and heating to boiling to coagulate the precipitate. The solution is made alkaline to phenolphthalein by the addition of barium hydroxide solution, and after cooling exactly neutralized with acetic acid. The precipitate is centrifuged, washed three times with 5-ml. portions of water, and centrifuged after each washing. It is transferred to a Kjeldahl flask, and the nitrogen determined as usual. The precipitate may also be filtered and washed on a filter paper, and the paper plus precipitate used for the nitrogen determination.

The tannin precipitation method, according to Ambler and Byall,[129] is applicable to white sugars also. Twenty-five grams of the sugar is dissolved in 100 ml. of water, and 5 ml. of 10 per cent tannin solution is added. The mixture is heated on the steam bath for 2 hours, and the precipitate filtered off on a quantitative filter paper. It is washed with ammonia-free water (p. 1078), and the paper with the precipitate transferred to an 800-ml. Kjeldahl flask. The nitrogen determination is carried out as described above for total nitrogen in white sugars (p. 1078). A blank is run and the result deducted from the nitrogen found previously. The protein nitrogen is expressed as parts per million.

Dědek and Ivančenko[130] recommend the tungstic acid reagent of Folin and Wu (p. 889) for separating the protein from the other forms of nitrogen.

Estimation of Amino Acid Nitrogen. Organic acids containing an amino group in the α position to the carboxyl yield with triketohydrindene hydrate, $C_6H_4(CO)_2C(OH)_2$, sold under the trade name of ninhydrin, an intensely blue or violet coloring matter.[131] A method for the determination of α-amino nitrogen has been based on this reaction by Riffart[132] and applied to sugar products by Ambler.[133] The principal α-amino acids in cane and beet products are aspartic and

[128] *Z. Zuckerind. čechoslovak. Rep.*, **46**, 691 (1921/22).
[129] *Ind. Eng. Chem., Anal. Ed.*, **4**, 34 (1932).
[130] *Ann. Fermentations*, **3**, 105 (1937).
[131] Ruhemann, *J. Chem. Soc.*, **97**, 2025 (1910).
[132] *Biochem. Z.*, **131**, 78 (1922).
[133] *Intern. Sugar J.*, **29**, 382, 437 (1927).

glutaminic acids, but the ninhydrin reaction is given also by their amides, asparagine and glutamine, and by peptides, peptones, albuminoids, and albumens. It is therefore a group reaction.

Ambler's method is carried out as follows. A stock solution of *l*-aspartic acid is prepared by dissolving 0.4749 g. of recrystallized acid in distilled water and diluting to 500 ml. This solution contains 100 mg. nitrogen per liter. Individual standards are prepared by diluting 5, 10, 15, etc., ml. of the stock solution to 100 ml. The stock solution and the standards may be preserved by covering the surfaces with toluene and keeping them in the refrigerator. The colorimetric comparisons must be made at a neutral reaction, and this is accomplished by means of a buffer solution, consisting of 2 volumes of a solution of 9.078 g. potassium dihydrogen phosphate per liter and 3 volumes of a solution of 11.876 g. secondary sodium phosphate ($Na_2HPO_4 \cdot 2H_2O$) per liter. The two phosphate solutions are kept separately and mixed immediately before using. The ninhydrin is used in the form of a 1 per cent solution, also prepared fresh just before each test. Sucrose gives a yellow color with ninhydrin, and in the examination of sugar products it is therefore necessary to add sucrose to the standards in order to compensate for the color developed in the sugar solution to be analyzed.

The material to be tested is used in the form of an approximately 50 Brix solution, and this is diluted, if necessary, with a 50 Brix solution of pure granulated sugar, so that not more than 25 mg. of amino acid nitrogen is present in 1 liter of solution. Blackstraps and refiner's sirup are diluted at a ratio of about 1 to 120.

One milliliter each of the unknown solution and of the standard solutions is pipetted into different test tubes, care being taken not to withdraw any toluene. To the standard solutions 1 ml. each of a 50 Brix pure sugar solution is added, and to the unknown 1 ml. of water; then to each of them 2 ml. of the mixed buffer solution, and finally 1 ml. of the 1 per cent ninhydrin solution. The solutions are well mixed by shaking. The tubes are placed in a wire basket and heated in a briskly boiling water bath for 30 minutes. At the end of this time the basket is lifted out with the tubes in it so that all may have exactly the same time of heating. The standard containing 5 mg. of nitrogen should have developed a decidedly blue-violet color. The tubes are cooled for ½ hour, the contents of each transferred to a Nessler cylinder, diluted with distilled water to 100 ml., and compared in the usual manner.

If the product to be analyzed is decidedly acid, owing to fermentation or other cause, the solution should be approximately neutralized prior to the test.

The ninhydrin method may be used, without modification, on white sugars also. The result is sometimes higher, sometimes lower, than the protein nitrogen. This is not surprising because, on the one hand, only a small proportion of the total nitrogen in protein is in the α-amino form, while on the other hand, not all the amino acids in sugar products are α-amino compounds. Since the tints of the ninhydrin compounds vary from one amino acid to the other, the tubes are sometimes difficult to match with the standards, but satisfactory approximations are usually possible.

Determination of α-Amino Nitrogen in the Presence of Large Quantities of Reducing Sugars. Ambler and Snider[134] later found that fructose in excess of 1 mg. and glucose in excess of 10 mg. in 2 ml. solution cause a brownish red color to develop, which masks the blue-violet of the ninhydrin test. In the analysis of sugar products containing large quantities of reducing sugars and small amounts of amino nitrogen, it is necessary therefore first to separate the amino acids from the sugars and then to apply the ninhydrin test. According to Lothrop and Gertler[135] this may be done by precipitating the amino acids with mercuric acetate, as proposed by Neuberg and Kerb.[136]

For the analysis of honey, 25 g. is diluted with 25 ml. of water, and the solution is mixed with 200 ml. of 95 per cent alcohol. The amino acids are precipitated by adding alternately from two burettes first 1 ml. of *N* sodium carbonate solution, followed by 1 ml. of *N* mercuric acetate solution, and so forth, until the white precipitate gradually changes to yellow or orange in color. After each addition the solution is tested with bromthymol blue paper, and if the solution is found to be acid, another milliliter of sodium carbonate solution is added. The final mixture should be slightly alkaline.

The precipitate is centrifuged, and washed three or more times with 20 ml. each of 80 per cent alcohol, the supernatant liquid being poured off after each centrifuging. The washed precipitate is then suspended in 50 ml. of water, and 5 drops of concentrated hydrochloric acid is added. The tube is immersed in a beaker of boiling water, and hydrogen sulfide is passed through for 10 minutes. The precipitate is centrifuged, and the supernatant liquid poured through a 7-cm. filter. The precipitate is stirred up with 20 ml. of water, 2 drops of concentrated hydrochloric acid is added, and hydrogen sulfide is passed through for a second time as before, to decompose the mercuric compounds completely. The precipitate is centrifuged again, and the supernatant liquid poured through the same filter. The precipitate is washed by stirring up with two 20-ml. portions of water, 1 drop of hydrochloric acid being added each time to prevent the formation of colloidal mercuric sulfide, and centrifuged as before.

[134] *Ind. Eng. Chem., Anal. Ed.*, **4**, 37 (1932).
[135] *Ind. Eng. Chem., Anal. Ed.*, **5**, 103 (1933).
[136] *Biochem. Z.*, **40**, 498 (1912).

The filtrate and washings are concentrated in vacuo to about 15 ml. and transferred to a small beaker. Two drops of phenolphthalein indicator is added, and dilute sodium hydroxide solution is added drop by drop until the solution is faintly alkaline. Very dilute hydrochloric acid is then added until the solution is just colorless. It should now be neutral to bromthymol blue paper. The solution is made up to 25 ml., and a 2-ml. aliquot, representing 2 g. of the original sample, is used for the determination of amino acid nitrogen by means of the ninhydrin test described above, without the addition of 1 ml. of sucrose solution.

If it is desired to exclude the amino nitrogen present in protein, this is first removed by diluting the honey with an equal volume of water, and adding 1 volume of 5 per cent bentonite suspension to 10 to 15 volumes of the honey solution. An aliquot of the protein-free solution is used for the amino nitrogen determination.

The method of Lothrop and Gertler should be used also in the analysis of the darker grades of soft refined sugars and of low-grade or badly deteriorated raw sugars. The protein may be removed, if desired, by the tannin treatment described on p. 1080.

The determination of amino nitrogen may at times be helpful in distinguishing between natural and artificial honeys, the latter being free from it unless it has been purposely added.[137]

Determination of Ammonia Nitrogen. This form of nitrogen cannot be determined by boiling with excess sodium hydroxide and titration of the ammonia evolved, as is usually done, because the acid amides in sugar products, as asparagine and glutamine, are partially decomposed and increase the ammonia found. Correct results are obtained by distilling with excess magnesium oxide under a partial vacuum at 40 to 50° C., absorbing the ammonia in standard sulfuric acid, and titrating back with standard alkali. However, the proteins are attacked by magnesium oxide even at this low temperature, and they must first be removed by treatment with tannin (p. 1080).

According to Dědek and Ivančenko,[138] the ammonia nitrogen can be determined more accurately and rapidly by the method of Folin and Bell.[139] Place 2 g. of sodium zeolite (Folin Permutit), which must be free from ammonia, in a 200-ml. volumetric flask; add 5 ml. of water and then 2 ml. of the sugar juice or solution. Wash the wall of the flask with another 5 ml. of water. Agitate the flask for 5 minutes. Dilute with 25 to 40 ml. of water, decant carefully from the zeolite, and

[137] Tillmans, *Z. Untersuch. Lebensm.*, 53, 131 (1927); Niethammer, *ibid.*, 58, 530 (1929).

[138] *Pub. inst. belge amélioration betterave*, 6, 337 (1938).

[139] *J. Biol. Chem.*, 29, 329 (1917).

repeat this washing process three times more. Liberate the ammonia from the zeolite in the flask by adding 10 ml. of 10 per cent sodium hydroxide solution, run in 5 ml. of Nessler's reagent, and dilute to the mark. Determine the ammonia colorimetrically by comparison with standards which have been prepared from ammonium chloride solution in the same manner as the solution tested. The method cannot be used upon dark-colored sugar products such as molasses because the color interferes with the determination.

Determination of Nitrogen in the Form of Acid Amides. In the original method of Schulze,[140] the acid amide group in asparagine, glutamine, etc., is split off by boiling with acid, and the resulting ammonium salt is decomposed with magnesia. But it was soon found[141] that this method gives low results when sugars are present in the product, because the sugars react with the ammonia under the formation of dark-colored nitrogenous condensation products from which the ammonia cannot be recovered by boiling with alkali. It is therefore necessary first to separate the ammonia and amides from the sugars. This is accomplished by precipitation with mercuric acetate, as in the method of Lothrop and Gertler for the determination of amino acids (p. 1082). Vondrák[142] uses the following procedure: The protein is first removed by precipitation with tannin, as described on p. 1080. To an aliquot of the filtrate, containing 25 to 30 mg. nitrogen, *N* mercuric acetate solution is added, at the rate of at least 1 ml. for each milligram of nitrogen. Then an equal volume, or better a slight excess of *N* sodium carbonate solution is run in with constant stirring. The precipitate is separated by centrifuging or filtration, washed, suspended in about 50 to 60 ml. of water, acidified with 5 ml. of sulfuric acid (1 : 1), and the mixture is boiled under reflux for 4 hours. It is nearly neutralized with sodium hydroxide, and an excess of magnesium oxide suspension is added. The mercury in the precipitate is converted into sulfide by the addition of a solution containing 50 per cent of sodium thiosulfate and 5 per cent of magnesium sulfate, equal in quantity to the volume of mercuric acetate solution used originally for precipitating the amides. The ammonia is distilled, under a vacuum at 40 to 50° C., into standard acid, and the excess acid titrated back with standard alkali. The result is the nitrogen in the form of ammonia and of acid amides; the latter represents one-half of the total nitrogen in the form of asparagine and glutamine, the other half being present in the amino form.

The ammonia originally present in the product is determined by

[140] *Landw. Versuchsstat.*, **26**, 254 (1881); **29**, 404 (1883).
[141] Kreusler, *Landw. Versuchsstat.*, **31**, 207 (1885).
[142] *Z. Zuckerind. čechoslovak. Rep.*, **51**, 261 (1926/27).

deproteinizing another portion of the sample, precipitating with mercuric acetate and sodium carbonate, and carrying out the distillation as before with excess magnesia in the presence of sodium thiosulfate and magnesium sulfate, but omitting acidification with sulfuric acid, boiling under reflux, and neutralization with sodium hydroxide. The ammonia nitrogen found in this determination is deducted from the nitrogen found previously, and the difference reported as nitrogen in the form of acid amides.

Determination of Nitrogen in the Form of Organic Bases. This group of nitrogenous compounds comprises betaine, choline, and a number of purine bases. Betaine is the most abundant one in beet products. In cane products the organic bases are of minor importance. They are precipitated by phosphotungstic acid or by potassium triiodide. The latter reagent has been used by Staněk[143] for the quantitative determination of betaine and choline. According to Vondrák[144] a solution of the sugar product is deproteinized with tannin (p. 1080), the filtrate evaporated to sirup consistency, and the residue dissolved in 20 ml. of a saturated solution of sodium chloride in 5 per cent sulfuric acid. A solution of 100 g. potassium iodide and 153 g. iodine in 200 ml. of water is added drop by drop as long as a brown precipitate forms. The mixture is allowed to stand for several hours in the refrigerator; the precipitate is then filtered off under suction on a filter paper in a small Büchner funnel, and washed five times with 2 ml. each of saturated sodium chloride solution. The nitrogen is then determined in the precipitate by the modified Kjeldahl method, applicable to betaine (p. 1077), and reported as nitrogen precipitated by potassium triiodide. For the separation of the betaine from the other bases the chemist is referred to Staněk's articles on this subject.

Method of Blood and Cranfield for Determining Betaine in Molasses.[145] This is a modification of Staněk's method. About 5 g. of the molasses is weighed into a beaker and dissolved in 50 ml. of water. The solution is clarified with the requisite amount of lead subacetate solution with constant stirring, and the precipitate is filtered off and washed. The filtrate and washings are acidified with 25 per cent sulfuric acid, and the lead sulfate is filtered off and washed. A few grams of zinc metal coated with metallic copper are added to the filtrate and washings, and the mixture is heated on the water bath for at least 4 hours to reduce the trimethylamine oxide present in molasses to tri-

[143] *Z. Zuckerind. Böhmen*, 28, 578 (1903/04); 29, 110 (1904/05); 31, 316 (1906/07); 34, 297 (1909/10); 37, 385 (1912/13); 40, 51 (1915/16).
[144] *Z. Zuckerind. čechoslovak. Rep.*, 46, 691 (1921/22).
[145] *Analyst*, 61, 829 (1936).

methylamine. The mixture is now transferred to a 500-ml. Kjeldahl flask, made alkaline with solid barium hydroxide, and distilled in a current of steam, to remove ammonia, dimethylamine, and trimethylamine. The residue in the flask is acidified with sulfuric acid, the barium sulfate filtered off, washed, and the filtrate and washings are made up to a definite volume. An aliquot of the solution containing 0.25 to 0.5 g. of the original molasses (5 to 50 mg. of betaine) is evaporated to 5 ml. A few drops of concentrated sulfuric acid are added, and the betaine is precipitated as the periodide by adding 2 ml. of a solution containing 20 g. of potassium iodide and 20 g. of iodine in 100 ml. The mixture is stirred; the betaine periodide is allowed to crystallize for 1 hour, collected on a Gooch crucible, and washed rapidly with three successive portions of 10 per cent sulfuric acid. The suction is stopped as soon as the wash liquid has passed through. The iodine solution adhering to the outside of the crucible is removed, and the precipitate is dissolved and washed into the original vessel with 95 per cent alcohol. When all the betaine periodide is dissolved (a little iodide-iodine solution being added if necessary) the solution is diluted with water and titrated with $N/20$ sodium thiosulfate solution, starch being used as indicator. Since 1 molecule betaine requires 5 molecules of iodine, the thiosulfate titer must be multiplied by 0.001171 to convert it into grams of betaine. The recovery of periodide is not quite complete, and a factor of 0.001181 was found experimentally. If the betaine periodide does not crystallize readily, owing to the peptizing effect of sugars, these must be destroyed by adding 2 ml. concentrated sulfuric acid to another aliquot of the solution and heating on a steam bath. The charred mass is extracted with hot water, the extracts evaporated to 5 ml., and the analysis then completed as described above.

Staněk's original method was found to give high results because the interfering bases are not removed. If betaine is to be determined in feeds prepared from dried beet pulp and molasses, the feed is first ground and extracted with water in a Soxhlet extractor.

Davies and Dowden[146] precipitate the betaine and other bases with 20 per cent phosphotungstic acid in 5 per cent sulfuric acid, recover the bases by treatment with barium hydroxide, remove asparagine, etc., by precipitation with mercuric acetate and sodium carbonate, and determine the betaine nitrogen in the filtrate by the Kjeldahl method. According to Blood and Cranfield this method is rather tedious.

Harmful Nitrogen. This is defined as the nitrogen in those substances that are not removed by the purification processes employed in the sugar factory, and hence pass into the final molasses, thereby re-

[146] *J. Soc. Chem. Ind.*, **55**, 175 (1936).

ducing the sugar yield. It is calculated by deducting from the total nitrogen that present in the form of protein, ammonia, and acid amides. The determination of harmful nitrogen thus requires a number of analytical operations. Of the total harmful nitrogen, that in the form of amino groups is considered the most objectionable, and various simple methods for its estimation have been introduced. The ninhydrin method, described on p. 1080, may be used for this purpose. Another method, due to Staněk and Pavlas,[147] makes use of the deep blue color which amino acids produce with solutions of copper acetate. The colorimetric standards are prepared from copper sulfate and cobalt ammonium sulfate and are based on the color given by glutamine with the copper acetate reagent. The result is designated as the " blue number." In a third method, due to Sørensen[148] and introduced into the sugar industry by Riehm,[149] the basic amino group is converted into a $N{=}CH_2$ group by treatment with formaldehyde, and the resulting free acid is titrated with alkali. This method has been simplified by Storck.[150] Unverdorben and Spielmeyer[151] have proposed to determine the nitrogen in the solution obtained when a sugar product is prepared for polarization by clarification with lead subacetate and filtration. The details of the different methods are omitted here because they are of interest primarily in sugar-factory control.

Determination of Nitrates and Nitrites. When appreciable quantities of nitrates and nitrites are present, these may be determined by the method of Schulze-Tiemann, based on the reduction to nitrogen monoxide, NO, by treatment with ferrous salt, and measurement of the gaseous oxide evolved. In the presence of mineral acid, both nitrates and nitrites are reduced; in the presence of acetic acid only nitrites.

A. *Nitrate plus Nitrite.* Stüber[152] recommends the following procedure for this determination. A 150-ml. round flask of resistance glass, provided with a two-hole stopper, is used. In one of the holes a funnel tube with a glass stopcock is inserted. The second carries a descending delivery tube, the other end of which is bent upward a short distance and placed in a trough filled with 20 per cent sodium hydroxide solution, so that the level of the liquid is above the outlet which is protected by a short piece of rubber tubing stretched over it. The solution to be analyzed is made alkaline, boiled to drive out the air, and poured into the

[147] *Z. Zuckerind. čechoslovak. Rep.*, 59, 129 (1934/35); 60, 46 (1935/36).
[148] *Biochem. Z.*, 7, 45 (1908).
[149] *Z. Ver. deut. Zucker-Ind.*, 85, 381 (1935).
[150] *Z. Ver. deut. Zucker-Ind.*, 88, 733 (1938); see also Janke, Holota, Mikschik and Sorgo, *ibid.*, 89, 379, 516 (1939).
[151] *Z. Ver. deut. Zucker-Ind.*, 89, 616 (1939).
[152] *Z. Untersuch. Nahr. u. Genussm.*, 10, 329 (1905).

funnel the stopcock of which is closed. Forty milliliters of a saturated solution of ferrous chloride and 40 ml. of 20 per cent hydrochloric acid are transferred to the flask, the stopper is replaced, and the mixture is boiled until all the air has been expelled and steam issues through the delivery tube. At this moment a eudiometer tube, filled with 20 per cent sodium hydroxide solution, is placed over the opening of the delivery tube in the trough, the stopcock in the funnel is opened, and the solution to be analyzed is run very slowly into the flask, boiling being continued. The gas evolved rises to the top of the eudiometer tube. Without drawing in air, the funnel is washed three times with 10-ml. portions of boiled 20 per cent hydrochloric acid, and the rinsings are also run into the flask. When the evolution of gas ceases the flame is removed for a while, boiling being resumed once more to drive over the last traces of gas. The lower end of the eudiometer tube is shifted to one side, a porcelain crucible is placed over it to close it, and the tube is transferred to a high glass cylinder filled with 20 per cent sodium hydroxide. When temperature equilibrium has been established, the levels inside and outside of the eudiometer are equalized, and the volume of the gas is read and reduced to 0° C. and 760 mm. pressure by the following formula:

$$V_1 = \frac{V\,(b - h)}{760\,(1 + \alpha t)}$$

where V_1 is the corrected volume, V the volume read at temperature t and pressure b, h the vapor tension of water at temperature t, and α the expansion coefficient for gases, $= 0.00366$. The corrected volume in milliliters, multiplied by 1.340, gives milligrams of NO, or if multiplied by 1.251, the milligrams of nitrogen in the form of nitrates and nitrites. Instead of a eudiometer and trough, a Schiff nitrometer may be employed to collect the nitrogen oxide.

B. *Nitrite Nitrogen.* According to Staněk,[153] this determination is carried out in the same way as that of nitrogen as nitrates plus nitrites, the only difference being that a solution of ferrous ammonium sulfate is substituted for the ferrous chloride, and acetic acid for the hydrochloric acid. The nitrogen in the form of nitrite thus found is deducted from the nitrogen in the form of nitrate plus nitrite, and the difference gives the nitrate nitrogen.

The reaction of nitrates with *m*-xylenol (1-hydroxy-2,4-dimethylbenzene) in strong sulfuric acid, leading to the formation of nitro *m*-xylenol, and the colorimetric estimation of the latter have been used

[153] *Z. Zuckerind. Böhmen,* **26,** 228 (1901/2).

by Blom and Treschow[154] for the determination of nitrates in soils and plants. Werr[155] has modified this method and applied it to the various parts of the sugar beet.

Nitrate and Ammonia Nitrogen in White Sugars. According to Ambler and Byall,[156] the nitrogen in the form of nitrates is best determined by reduction to ammonia by means of Devarda's alloy (10 parts copper, 9 parts aluminum, 1 part zinc). Two and one-half grams of the sugar is dissolved in 250 ml. of magnesia water (see p. 1078) in an 800-ml. Kjeldahl flask. One gram of Devarda metal, ground to pass a 50-mesh screen, 50 ml. of a solution containing 400 g. pure sodium hydroxide per liter, and a teaspoonful of magnesium oxide are added, and the flask is immediately connected with the condenser and receiver. A similar solution is prepared at the same time, but without the addition of the alloy, and connected with another condenser and receiver. After the evolution of hydrogen in the first flask has subsided, which requires 30 minutes or more, the mixtures are slowly heated to boiling and distilled, as described for the determination of total nitrogen in white sugars (p. 1078), until 200 ml. of distillate has been collected. The ammonia is then determined colorimetrically with Nessler's reagent. Blank determinations are carried out without sugar, and the nitrogen found is deducted from that obtained in the distillations with and without Devarda metal, respectively. The corrected nitrogen, found in the distillation without the metal, is that originally present as ammonia. When this is deducted from the nitrogen obtained in the presence of the alloy, the difference represents the nitrogen in the form of nitrates and nitrites. The nitrite nitrogen is determined in another portion of the sample by the method given below, and a correction is applied to obtain the nitrate nitrogen. The nitrite nitrogen usually amounts to so little, in comparison with the nitrate nitrogen, that the correction can in most cases be neglected.

The distillates obtained in this method usually give a greenish color when the Nessler reagent is added, but this does not interfere with the development of the color due to ammonia. The tubes can readily be matched with the standards by viewing both through a yellow light filter, Wratten K-3, No. 9.

Determination of Nitrites in White Sugars. For this determination the extremely sensitive reaction of Griess[157] is employed.[158] Nitrous

[154] *Z. Pflanzenernähr. Düngung Bodenk.*, **13A**, 159 (1929).
[155] *Z. Ver. deut. Zucker-Ind.*, **87**, 119 (1937).
[156] *Ind. Eng. Chem., Anal. Ed.*, **4**, 34 (1932).
[157] *Ber.*, **12**, 426 (1879). See also Ilosvay de Nagy Ilosva. *Bull. chim.* [3], **2**, 347 (1889).
[158] **Ambler and Byall,** *Ind. Eng. Chem., Anal. Ed.*, **4**, 34 (1932).

acid gives with *p*-aminobenzenesulfonic acid (sulfanilic acid) and α-naphthylamine a beautiful red azo dye. Even at extreme dilutions of nitrous acid a decided pink is observed. The sulfanilic acid is used in the form of a solution containing 0.5 g. in 150 ml. of 20 per cent acetic acid. Two-tenths of a gram of α-naphthylamine hydrochloride is dissolved, by heating, in another 150 ml. of 20 per cent acetic acid. A stock solution of nitrite is prepared by dissolving 0.1097 g. of pure, dry silver nitrite in about 20 ml. of hot water, adding 0.1 g. of sodium chloride, shaking until the silver chloride flocculates, and diluting to 1 liter. When the silver chloride precipitate has settled, 10 ml. of the clear solution is pipetted off and diluted to 1 liter. This final solution contains 0.0001 mg. nitrite nitrogen in each milliliter. The comparison standards are made by dissolving 20 g. each of pure, nitrite-free sucrose in water in Nessler tubes, adding to each increasing quantities of the stock solution of nitrite, and filling up to 100 ml. Twenty grams of the sugar to be tested is dissolved in another Nessler tube and made up to 100 ml. Then 2-ml. portions each of the two reagents are added to all of the tubes, and after 30 minutes' standing the unknown is compared with the standards. If the unknown solution shows a yellow or brownish color, a few drops of a very dilute caramel solution are added to each of the standards, to compensate for it. Colored sugars and juices cannot be analyzed by this method, however, because the color of the dye would be completely masked.

The white sugars examined by Ambler and Byall were found to contain a maximum of 30 p.p.m. of nitrate nitrogen, and of 12 p.p.m. of ammonia nitrogen, but not over 0.25 p.p.m. of nitrite nitrogen.

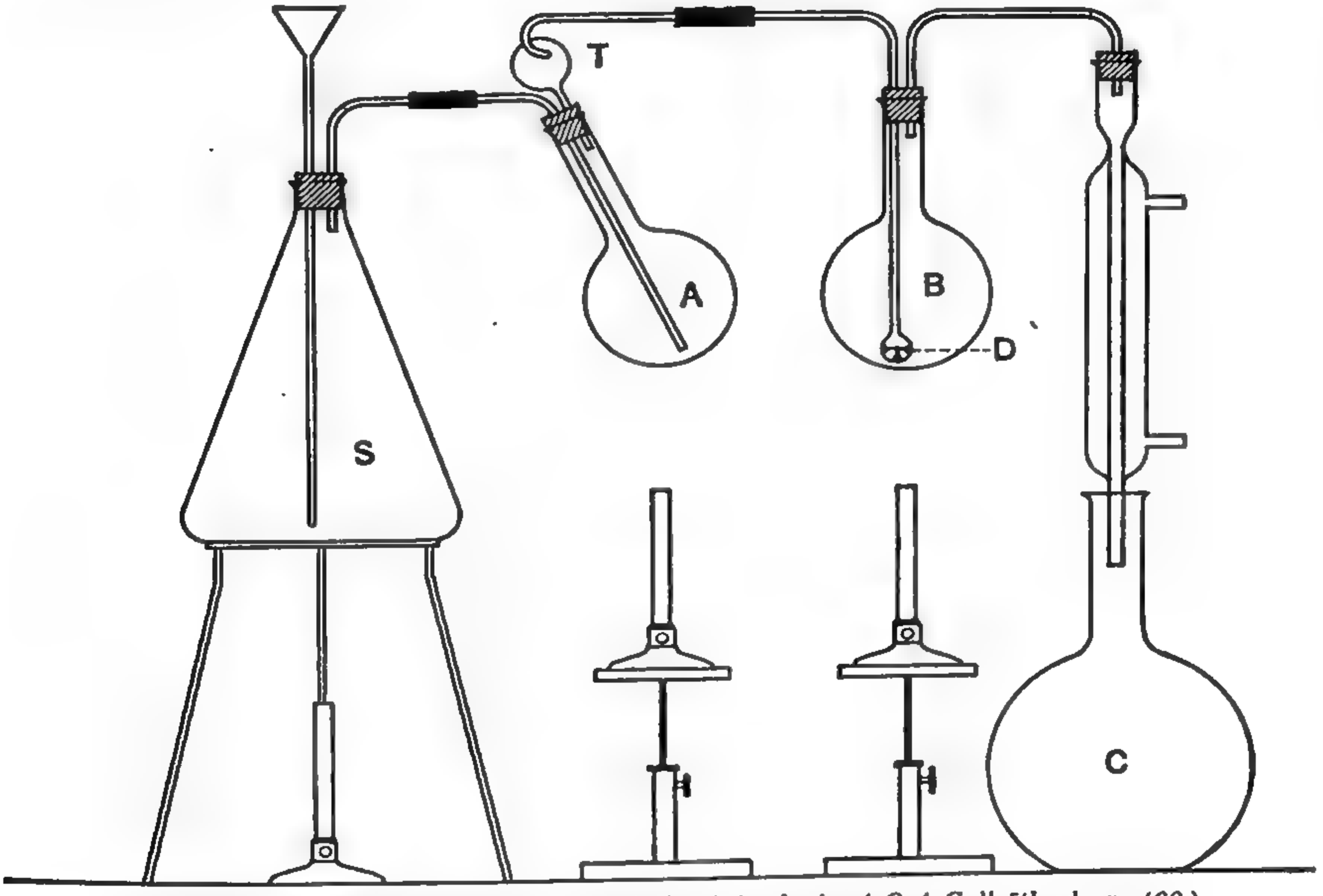
Five samples of refined cane sugar analyzed by the Carbohydrate Research Division of the Bureau of Chemistry and Soils showed an average of 3.9 p.p.m. of total nitrogen and 0.8 p.p.m. of nitrate nitrogen; 57 samples of high-grade beet sugar showed an average of 27.6 p.p.m. of total nitrogen and 2.9 p.p.m. of nitrate nitrogen.

Determination of Formic Acid. Some countries prohibit the addition of formic acid to foodstuffs as a preservative. Zerban[159] has found that certain genuine sugar products may contain considerable quantities of this acid, formed by the decomposition of sugars through heating, especially in the presence of alkali. Bone black and decolorizing carbons catalyze this decomposition. For this reason filtered refinery sirups may contain as much as 0.8 per cent of formic acid. Raw sugar molasses contains only about 0.15 per cent, cane sirups 0.01 per cent, raw cane sugars up to 0.03 per cent, soft refined sugars up to 0.15 per cent, but granulated sugars less than 0.002 per cent. The determination

[159] *J. Assoc. Official Agr. Chem.*, **15, 355 (1932).**

is carried out by the method of Fincke, which has been adopted by the Association of Official Agricultural Chemists in the following form:[160]

The apparatus is shown in Fig. 316. It consists of a steam generator *S*; a 300-ml. flask *A* for the sample; a spray trap *T*; a 500-ml. flask *B*, containing a suspension of 2 g. barium carbonate in 100 ml. of water; a condenser, and a 1-liter volumetric flask, *C*. *D* is a Folin ammonia tube with a number of small holes in the bulb to break the vapor into small bubbles.



(Reproduced with permission from "Methods of Analysis, A.O.A.C.," 5th ed., p. 466.)

FIG. 316. Apparatus for determination of formic acid.

Fifty grams of the sugar or sirup is transferred to flask *A*, and 50 ml. of water and 1 g. of tartaric acid are added. The contents of flasks *A* and *B* are heated to boiling, and steam from the generator *S* is passed through the apparatus. The burners under *A* and *B* are regulated so that the volume of liquid in both remains constant. When 1 liter of distillate has been collected the apparatus is disconnected, the contents of flask *B* are filtered hot, and the barium carbonate is washed with hot water. The filtrate and washings should measure about 150 ml. Ten milliliters of a filtered sodium acetate solution, containing 50 g. in 100 ml., is added, then 2 ml. of 10 per cent hydrochloric acid (30 ml. concentrated acid plus 70 ml. water), and finally 25 ml. of a solution of 100 g. mercuric chloride and 150 g. sodium chloride in a total volume of 1 liter. The

[160] "Methods of Analysis, A.O.A.C.," 5th ed., pp. 466–467, 1940.

mixture is thoroughly stirred, and the flask or beaker immersed in a boiling-water bath for 2 hours. The precipitate of mercurous chloride is filtered on a Gooch crucible, washed well with cold water, and finally with a little alcohol. It is dried in a boiling-water oven for 30 minutes, cooled, and weighed. The weight of the precipitate, multiplied by 0.0975, gives the weight of the formic acid (HCOOH). If more than 1.5 g. mercurous chloride is obtained, the analysis is repeated with a smaller quantity of sample. The reagents are tested for formic acid by running a blank with 150 ml. of water, 1 ml. of 10 per cent barium chloride solution, 2 ml. of the dilute hydrochloric acid, 10 ml. of the sodium acetate solution, and 25 ml. of the mercuric chloride solution. The weight of the mercurous chloride obtained is deducted from that found in the previous determination. If the product contains considerable acetic acid, besides formic, enough barium carbonate must be placed in flask *B* so that at least 1 g. remains at the completion of the distillation. Molasses often foams badly, and it is then necessary to reduce the amount of the sample to 20 or even 10 g., and to use a 500-ml. flask.

Wax in White Sugars. Observations by Bardorf[161] have indicated that the wax covering on the rind of the sugar cane is not completely removed by the manufacturing and refining processes, and that traces of it pass into the granulated sugar. The flocculation sometimes noted in carbonated beverages has been attributed to wax present in the sugar from which they were prepared. Although this has not been definitely proved, some refiners endeavor to remove the wax as completely as possible, and test the sugars for wax. Two methods are in use for this purpose.

Extraction with Chloroform. In the method of the Java Sugar Experiment Station,[162] 50 g. of sugar is weighed into an extraction thimble, which is then closed with a wad of fat-free cotton. The sugar is extracted with chloroform in a Soxhlet extraction apparatus, the flask being weighed dry before the chloroform is added. When the extraction is complete the extract is concentrated in the flask to 25 ml. and transferred quantitatively to a separatory funnel the stopcock of which is lubricated with graphite. The chloroform extract is shaken out three times with 25 ml. of water each, to remove sugar and other water-soluble substances, and is then filtered through a dry filter paper, in which a layer of anhydrous sodium sulfate has been placed, into the weighed extraction flask. The filter is washed three times with 10 ml. each of chloroform. The extract and washings are distilled until about 5 ml. remains, and then evaporated on the water bath until the odor of chloroform has disappeared. The residue is dried to constant weight in

[161] *Can. Chem. Met.*, 11, 231 (1927).

[162] "Methoden van Onderzoek bij de Java-Suikerindustrie," 6th ed., p. 380, 1931.

a water oven, and the result is expressed in percentage on the original weight of the sugar.

Extraction with Acetone.[163] In this procedure the water-soluble crystalloids are first removed by dialysis. The dialyzer is made by pouring collodion into a 500-ml. cylinder, distributing it evenly over the surface by rotating the cylinder, draining for a few minutes, and allowing to dry. The membrane is carefully loosened at the edges and pried away from the cylinder wall. Water is run in between the sack and the wall of the cylinder, and the sack gradually pulled out. It is tested for leaks by filling it with water. One hundred grams of the sugar is dissolved in about 100 ml. of water, the solution is washed into the collodion sack, and enough water is added so that the sack may be closed and the top tied with a string. The sack is immersed in water in a bucket, and the sugar solution dialyzed in running water for 72 hours, or until the run-off water is completely free of sugar. The sack is emptied into a porcelain dish, thoroughly washed, and the solution evaporated to dryness on a water bath. The residue is rubbed with 50 ml. of acetone, the solution filtered into a tared dish, and the filter washed with acetone. The filtrate and washings are evaporated to dryness, the dish is placed in an oven heated to 100° C., and the dried residue is weighed. A blank is run on an equal quantity of acetone, the result being deducted from that obtained in the test on the sugar. The acetone-soluble wax is expressed in parts per million.

Determination of "Gums." Some of the colloids present in sugar products are precipitated by strong alcohol, and these are commonly referred to as "gums." The precipitate consists principally of pectins, hemicelluloses, and dextrins, but contains also nitrogenous substances, ash, and occasionally dextran and similar polysaccharides formed by the action of bacteria. The gums increase the viscosity and retard filtration, and precipitation with alcohol offers a rapid means for approximate determinations. The method has been carefully studied by Ruff and Withrow,[164] who recommend the following procedure. Sugars are dissolved in an equal weight of water; sirups, molasses, or juices are diluted or concentrated to about 50 per cent solids. Ten milliliters of the solution, which has been centrifuged or filtered through a Gooch crucible to remove suspended matter, is pipetted or weighed into a small beaker or Erlenmeyer flask, 0.5 ml. of concentrated hydrochloric acid is added and then, with constant stirring, 50 ml. of 95 per cent alcohol. Methanol or denatured alcohol may be used, but they do not give as consistent results as ethyl alcohol. If the gum

[163] Private communication from B. H. Varnau, Imperial Sugar Company.

[164] *Ind. Eng. Chem.*, 14, 1131 (1922).

content of the product is low, the volumes of solution, of hydrochloric acid, and of alcohol are doubled. The mixture is allowed to stand for 15 minutes and then filtered through a Gooch crucible with a mat of at least 0.2 g. asbestos. It is not necessary to weigh the crucible before the filtration. The alcoholic solution is decanted through the crucible; when it has all passed through, the precipitate is transferred to the filter with 95 per cent alcohol, and thoroughly washed with it. A little hydrochloric acid may be added to the wash alcohol to speed up filtration. The crucible is dried at 100 to 105° C. to constant weight, 1 hour usually being sufficient. It is then ignited in a muffle furnace and reweighed. The difference between the two weights is reported as gums. In accurate work the loss in weight when the crucible and asbestos alone are ignited after drying at 100° C. should be determined and corrected for. A correction may be applied also for the nitrogenous compounds present in the precipitate, by determining the nitrogen in another precipitate prepared in the same way, multiplying the nitrogen by the empirical factor 6.25, and deducting the weight of hypothetical protein thus found from that of the ash-free gums.

Beet molasses and other beet products, when analyzed by this method, sometimes give very sticky precipitates which are difficult to handle. Choppin and Withrow[165] found that in such cases the quantity of hydrochloric acid must be increased, 1 to 2 ml. of the acid usually proving sufficient. The alcohol concentration in the mixture must be held within narrow limits, 60 to 65 per cent by weight, and methyl alcohol may not be substituted for the ethyl alcohol. The results obtained by this modified method are sufficiently exact for control purposes.

DETERMINATION OF COLLOIDS

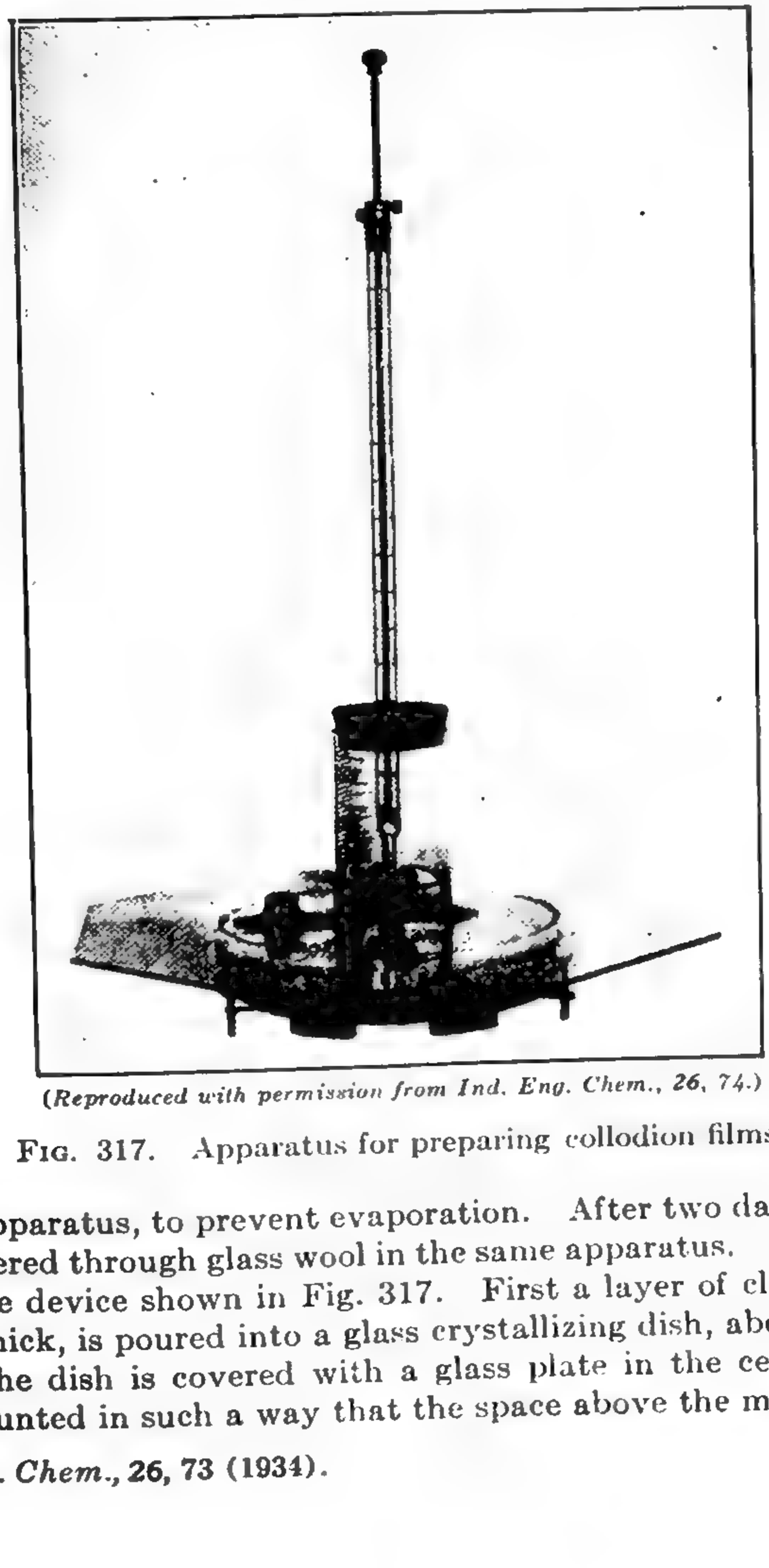
Colloids play an important part in sugar manufacture. Their removal is one of the principal aims in the clarification of beet or cane juices, because they increase the viscosity of the products and thus affect boiling and centrifuging. They are also adsorbed on sugar crystals during their growth, and cause the sugar to be off-color and dull in appearance. Various methods have been proposed for the determination of colloids in sugar products. Some of these are direct methods of separation, such as dialysis, ultrafiltration, or centrifuging. Dialysis, being tedious and difficult both to standardize and to control, has not been used to any extent. Centrifuging requires very high speeds, obtainable only with the ultracentrifuge, and has not been tried on sugar

[165] *Facts About Sugar,* **24, 446 (1929); private communication from J. R. Withrow.**

products. Ultrafiltration, however, has been used with considerable success.

Ultrafiltration Method of Dawson. This method is described by Paine, Gertler, and Lothrop as follows:[166]

The filter membranes are prepared from " Astoria Soluble " nitrocellulose, which is first thoroughly mixed and dried. Fifty grams is dissolved in a mixture of 600 ml. of absolute alcohol and 600 ml. of absolute ether in a hermeti-



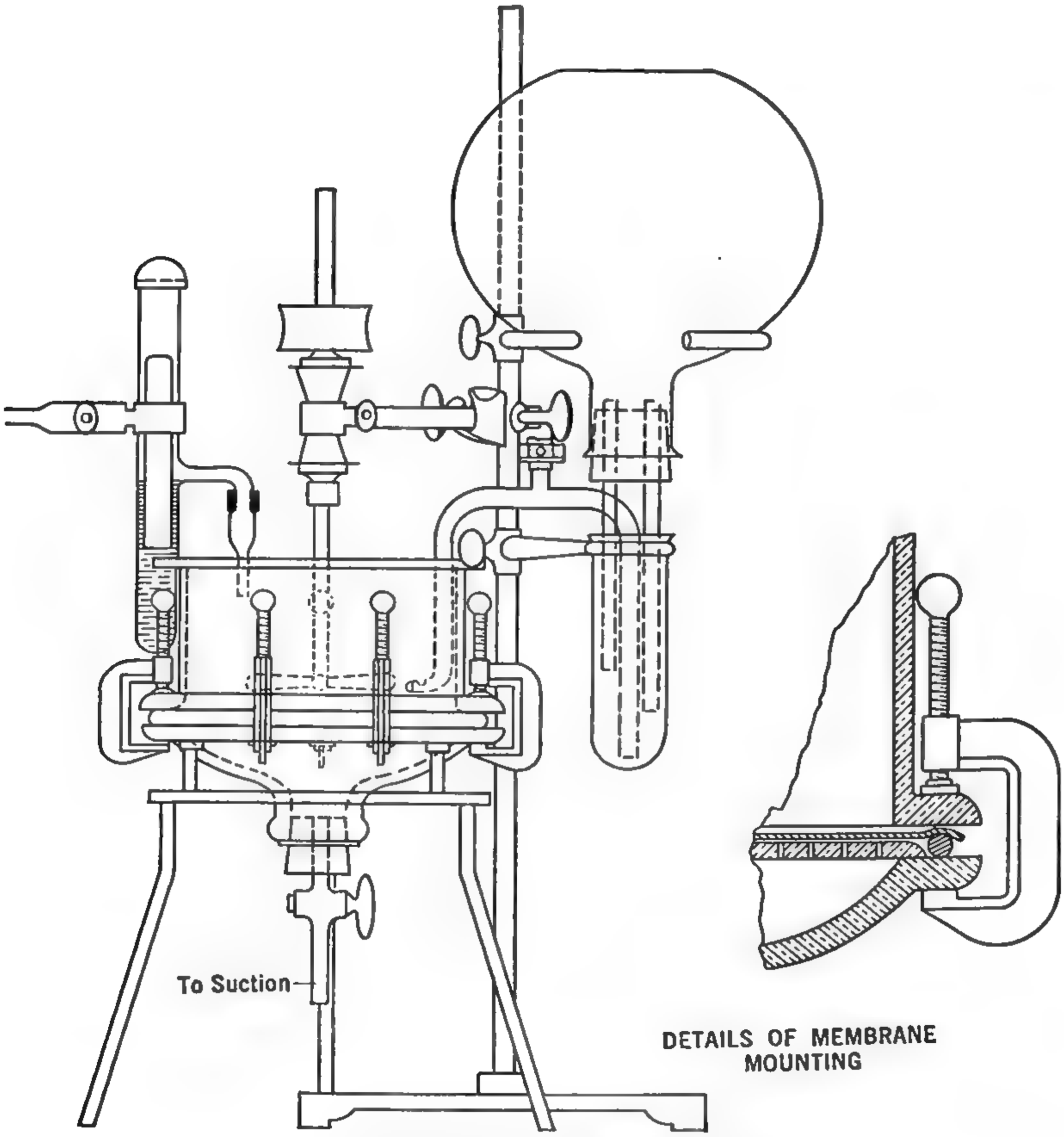
(*Reproduced with permission from Ind. Eng. Chem.*, 26, 74.)

FIG. 317. Apparatus for preparing collodion films.

cally sealed apparatus, to prevent evaporation. After two days' standing the solution is filtered through glass wool in the same apparatus. The membranes are cast in the device shown in Fig. 317. First a layer of clean mercury, at least 1 inch thick, is poured into a glass crystallizing dish, about 9.5 inches in diameter. The dish is covered with a glass plate in the center of which a burette is mounted in such a way that the space above the mercury is practi-

[166] *Ind. Eng. Chem.*, 26, 73 (1934).

cally air tight. Forty milliliters of the nitrocellulose solution is run from the burette onto the mercury surface by means of a tight-fitting plunger. After 30 minutes' standing the cover is lifted from the dish and supported at a definite distance above it, to permit gradual evaporation of the solvent. After another 30 minutes distilled water is poured over the film to harden it.



(Reproduced with permission from Ind. Eng. Chem., 26, 74.)

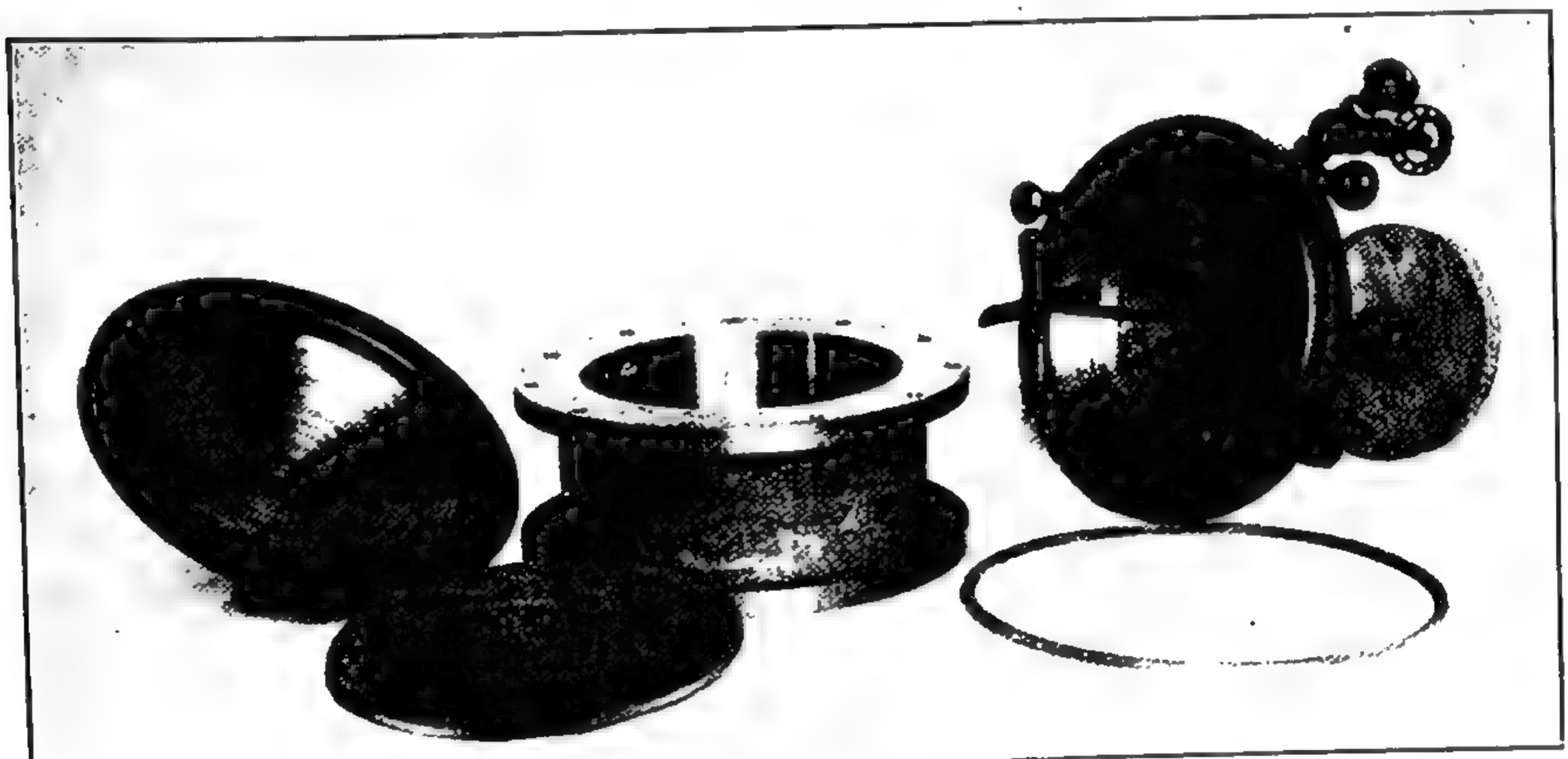
FIG. 318. Vacuum apparatus for ultrafiltration.

It is then carefully removed, and stored for several days before use in distilled water to which a small quantity of toluene has been added to prevent mold growth. In order to obtain comparable results, the membranes should be prepared in a room of constant temperature and humidity. They must be touched only at the edges when handled.

Collodion membranes always adsorb a certain quantity of colloids from

their solutions, and it is therefore necessary in quantitative work first to saturate the adsorptive capacity of each film by filtering through it, under suction, 1 liter of a solution containing 200 g. of the type of product to be analyzed and high in colloids. The porosity of each film is then measured by passing through it 1 liter of distilled water, at a constant absolute pressure of 20 inches of mercury (about 10 inches vacuum). The water rate of the films prepared as described will vary from about 130 to 180 ml. in 1 hour. The water rate must be redetermined after each use, to detect leaks which may have developed; it gradually decreases after each use, and then becomes practically constant.

The ultrafiltration apparatus, Fig. 318, consists of a glass cylinder, about 7 inches in diameter, with a heavy lower rim, and an inverted desiccator cover, with an outlet at the bottom. A perforated Monel metal plate, covered



(*Reproduced with permission from Ind. Eng. Chem., 26, 75.*)

FIG. 319. Pressure apparatus for ultrafiltration, showing separate parts.

with a 100-mesh Monel metal screen, is placed between the two. The membrane is placed on top of the screen, and a rubber gasket is put around the filter element. An air-tight seal is obtained by means of six clamps around the rim. The cylindrical vessel is covered with a glass plate, divided into halves, and provided with openings for the stirrer, the toluene dripping device, and the siphon for the wash water. The toluene dripping device is actuated by a plunger connected with a clock mechanism, to deliver toluene at a constant rate during the ultrafiltration. The siphon is also arranged so as to deliver the wash water at the same rate as liquid is withdrawn by the filtration.

Two hundred grams of the product to be analyzed is dissolved in distilled water to a volume of 1 liter, and the solution is strained through a closely woven linen cloth to remove coarse suspended matter. It is then washed into the apparatus, the stirrer and the toluene dripping device are started, and the

stopcock to the vacuum line is opened. When all but a small, definite portion of the solution has passed through the filter, washing is begun, the volume of the solution being kept constant at about 150 ml. Washing is continued day and night until the filtrate is found free from sugar by the α-naphthol test. The operation usually requires several days, but a number of ultrafiltration units can be run at one time. Time may also be saved by using a pressure outfit, depicted in Fig. 319, instead of the vacuum apparatus. It is similarly constructed but made entirely of Monel metal. It can also be equipped with an automatic washing device, but it is simpler to release the pressure intermittently and add small quantities of water and toluene at a time until the filtrate is free from sugar.

When the ultrafiltration is completed the liquid remaining behind in the vessel is removed, concentrated at a low temperature, and finally dried to constant weight at 40° C. under a vacuum of at least 29 inches. The results can be duplicated within 2 per cent of the total.

Ultrafiltration equipment, including membranes, is also available commercially and may be adapted for colloid determinations in sugar products.

Reversible and Irreversible Colloids. The colloids obtained by ultrafiltration may be separated into two fractions. During the operation of the ultrafilter a part of the colloids flocculates, owing to the removal of protective colloids. When the flocs are filtered off and dried, these colloids form a pulverulent mass containing a large proportion of ash, and do not redissolve in water. They are therefore classed as irreversible colloids, of the suspensoid or hydrophobic type. The colloids remaining in the filtrate from the irreversible portion give upon drying lustrous scales of a gummy nature, consisting principally of organic matter. They redissolve in water unless they have been heated too long. This fraction represents the reversible colloids, of the emulsoid or hydrophilic type. In the case of cane or beet products the two fractions can be separated easily by simple filtration as described. But with honeys the line of demarcation between reversible and irreversible colloids is less distinct, and it is better to separate the flocs by centrifuging at 2000 r.p.m. and washing the precipitate with three 50-ml. portions of water, followed by centrifuging after each addition of water.

Determination of the Gold Number. The gold number is a measure of the quantity of protective colloids present, and hence of the highly hydrated emulsoids. It has been defined by Zsigmondy as the maximum number of milligrams of protective colloid that may be added to 10 ml. of a colloidal gold solution without preventing a change of the red color to violet by 1 ml. of a 10 per cent solution of sodium chloride.

The colloidal gold solution is prepared[167] by heating 120 ml. of conductivity water (specific conductance 1.2×10^{-6} or better) in a thoroughly cleaned vessel of resistance glass, adding 2.5 ml. of a solution of gold chloride (6 g. $AuCl_3 \cdot HCl \cdot 3\ H_2O$ dissolved in conductivity water to 1 liter), and then 3.5 ml. of 0.18 *N* potassium carbonate solution. The solution is well stirred, heated to 100° C., and removed from the heater. Then 0.3 per cent solution of formaldehyde (0.3 ml. formalin and 100 ml. water) is added drop by drop, with thorough stirring after each addition, until the solution begins to show a faint red tint. Further additions of formaldehyde are now made only after a further change in color is no longer produced by a previous drop. A deep red, extremely clear solution is thus obtained by using in all about 2 ml. of the formaldehyde solution.

For the determination of the gold number of a sugar or sugar product, a solution of definite concentration is prepared, and varying volumes of it are pipetted into a series of small beakers. In each beaker the solution is mixed with 10 ml. of the colloidal gold solution. After 3 minutes, 1 ml. of a 10 per cent sodium chloride solution is added, and the mixture is well stirred. The gold number lies between the two volumes of sugar solution one of which produced a color change while the other did not. In a second series of tests the differences between the volumes of the sugar solution are narrowed down, and the exact gold number is represented by the volume of solution which just failed to change the color from red to violet. Several granulated sugars examined by Paine, Badollet, and Keane[168] gave gold numbers from 2600 to 3400 mg., and samples of beet molasses values between 800 and 1000 mg. The method gives good results with sugars, but suffers in precision with dark-colored products like molasses.

Colloid Determination by the Dye Test. This method, devised by Badollet and Paine,[169] is, like the gold number determination, much more rapid than ultrafiltration, and is widely used in the cane-sugar industry. It is based on the fact that the colloids in cane and beet products carry a negative electric charge. This charge is neutralized by the addition of a known amount of a positively charged colloid, causing complete flocculation of the colloid complex when the isoelectric point is reached. Although the principle of this procedure is quite different from that of ultrafiltration, there is a close correlation between the results of the two methods. The dye night blue has been found to be most serviceable as the positive colloid, producing very rapid

[167] Sheppard and Elliott, *Ind. Eng. Chem.*, 13, 699 (1921).
[168] *Ind. Eng. Chem.*, 16, 1252 (1924).
[169] *Intern. Sugar J.*, 28, 23, 97, 137, 497 (1926); *Louisiana Planter*, 79, 121 (1927).

flocculation and settling of the precipitate. One gram of the powdered dye is dissolved in distilled water and diluted to 1 liter. The solution should be prepared fresh once a week, on account of possible change in the electric charge.

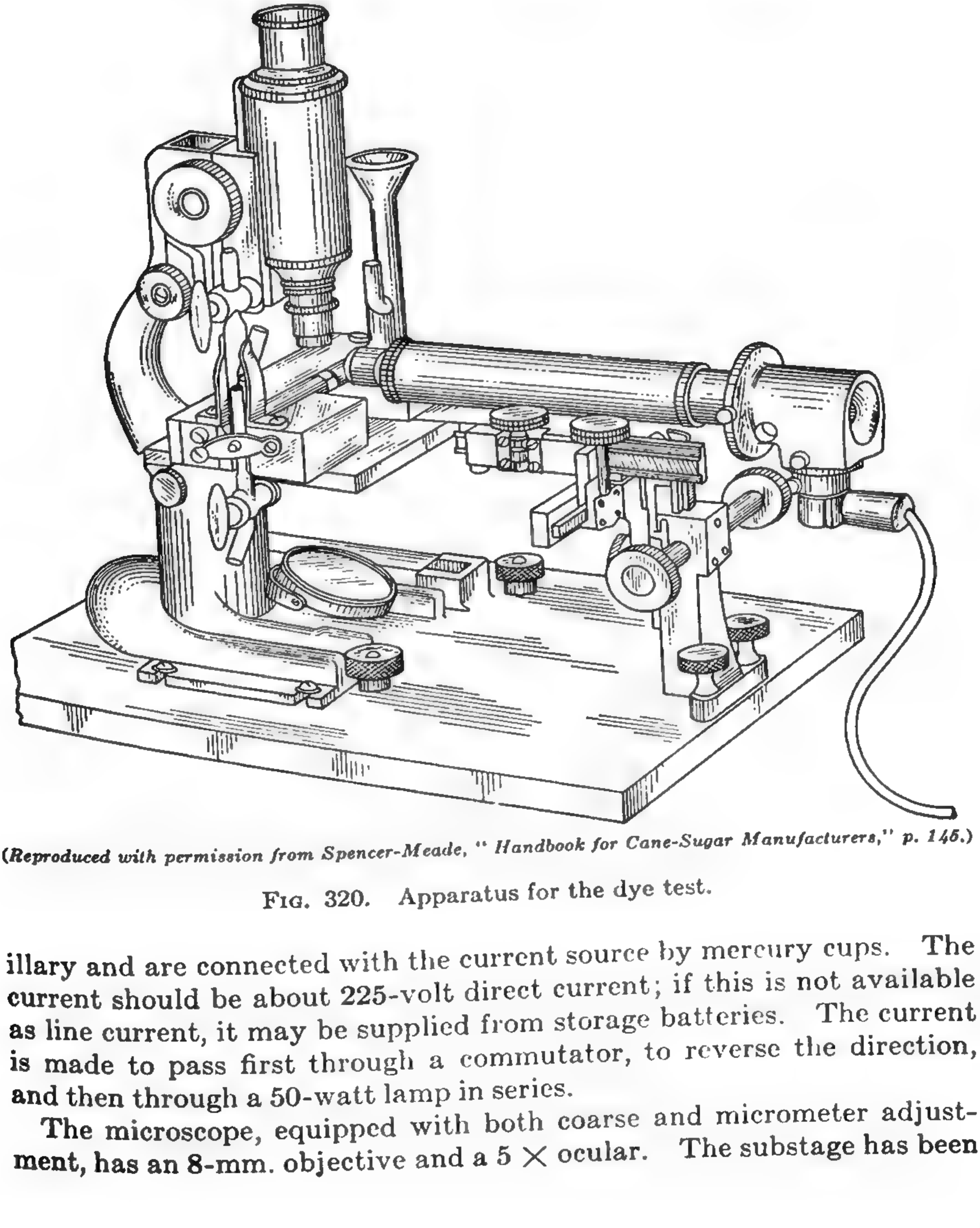
The results of the dye test vary with the *p*H of the solution to be examined. It is therefore necessary, in order to obtain comparable results, to adjust all the products in a series to the same *p*H. The addition of alkali should be avoided as far as possible, because it is likely to cause partial flocculation of the colloids present. For this reason it is best to select a fairly low *p*H value as standard. A figure of 5.2 is satisfactory for all the products of the raw cane sugar factory, but if comparison with the mixed juice is to be omitted, a standard of *p*H 6.0 may be used. This figure is also recommended for refinery products. Beet-sugar products have not been studied extensively; they are best adjusted to phenolphthalein neutrality. A 0.05 *N* solution of hydrochloric acid is used for the *p*H adjustment, and 0.05 *N* sodium hydroxide if it should be found necessary.

An approximate measure of the colloids may be obtained by preparing a number of tall tubes in each of which is placed 25 ml. of a solution of the sugar product, containing about 100 mg. of solids. To each of the tubes are added varying quantities of the night blue solution, always diluted further to 25 ml. The tubes are shaken, allowed to stand for a few minutes, and then examined in transmitted light. Those tubes which show the largest flocs and the most rapid settling indicate the range within which the isoelectric point lies.

Ultramicroscopic Cataphoresis Apparatus. For a more exact determination of the isoelectric point, the cataphoresis, that is the migration of the colloidal particles under the influence of an electric current, is observed by means of an ultramicroscope. Particles carrying a negative charge migrate to the positive electrode, and vice versa. When a colloid of opposite charge is added the migration velocity of the particles is reduced. At the isoelectric point migration stops completely, and an excess of the oppositely charged colloid reverses the direction of the migration.

The equipment used is shown in Fig. 320. The cataphoresis cell, which is securely fastened to a hardwood block on the stage of the microscope, is made of a piece of capillary tubing. A piece in the center of the capillary is filed to a flat surface on the side facing the light source and also on the top, facing the microscope objective at a right angle to the other surface. The upper plane surface should be filed as close as possible to the capillary and covered with a thin cover glass cemented with Canada balsam to present an optical surface. A glass

reservoir is sealed to each end of the capillary tube. One of the reservoirs, about 3 inches high, widens to a funnel, open on top. The other forms a T with the capillary tube; the upper leg is about 2 inches high. Both legs are provided with glass stopcocks which should be well greased, and kept tightly closed when a cataphoresis observation is being made. Platinum electrodes are fused in at both ends of the cap-



(*Reproduced with permission from Spencer-Meade, "Handbook for Cane-Sugar Manufacturers," p. 145.*)

FIG. 320. Apparatus for the dye test.

illary and are connected with the current source by mercury cups. The current should be about 225-volt direct current; if this is not available as line current, it may be supplied from storage batteries. The current is made to pass first through a commutator, to reverse the direction, and then through a 50-watt lamp in series.

The microscope, equipped with both coarse and micrometer adjustment, has an 8-mm. objective and a 5 × ocular. The substage has been

removed. The capillary is illuminated by a strong beam of light, at right angle to the direction of observation. The illuminating unit may be adjusted in three directions by rack and pinion and focused on the capillary. The microscope is focused on a point, in the vertical central plane of the capillary, at which the electro-osmotic movement of the liquid is zero. This point is at 0.293 times the radius of the capillary below its upper wall. To focus on the upper wall, the cell is filled with a colloidal solution, and the objective is lowered until the colloidal particles first become visible. If the radius of the capillary is known, the objective is lowered the calculated amount by means of the micrometer adjustment. If the radius is not known, it may be determined in the following manner. A suspension of powdered granulated sugar in sugar-saturated alcohol is placed in the capillary, and the objective is focused rapidly on the highest visible particle, before the suspension has had time to settle. The reading on the vernier of the micrometer is noted. After the suspension has settled, the objective is focused on the lowest particle in the capillary, and the micrometer read again. The difference between the two readings is divided by 2 and multiplied by 0.293, and the milled head is set at this distance below the upper wall of the capillary.

Dye Test Procedure for Raw and Washed Raw Sugars. Five grams of the sugar is dissolved in 25 ml. of distilled water, and the solution is filtered through a 100-mesh screen, which is then washed with small quantities of water. The filtrate and washings are diluted to 100 ml. in a 600-ml. beaker. The *p*H of the solution is then adjusted to the desired figure, e.g., *p*H 6.

Ten milliliters of the standard night blue solution is added to the 100 ml. of the sugar solution, thoroughly mixed with it, and the mixture poured into the funnel of the cataphoresis cell until the liquid overflows through the open stopcock at the top on the other side. The cock is now closed and an observation made with the perfectly adjusted instrument. There should be no progressive movement (neglecting Brownian movement) toward either electrode while the current is off. Any such progressive movement is due either to leaks or to air bubbles, which defects must be corrected before the current is turned on. The switch is now closed. If the particles move toward the positive electrode they still carry a negative charge. In this case the stopcocks are opened, the solution is drained into the original beaker, more dye solution is added, and after mixing well the solution is retested in the cataphoresis cell. This process is repeated until the colloid flocs fail to move progressively toward either anode or cathode when the current is turned on. It is advisable to reverse the direction of the current dur-

ing an observation every few seconds in order to avoid the disturbing effect of polarization of the electrodes. When the isoelectric point has been reached, the total number of milliliters of dye solution used is noted. As a check, it is well to add a little more dye solution and to make sure that the flocs now move toward the cathode. The number of milligrams of dye in the volume of dye solution required to reach the isoelectric point is now divided by the number of grams of sugar in the sugar solution, and the result multiplied by 100. This gives the dye number of the sugar.

Example. The 5 g. of sugar required 20 ml. of night blue solution, equal to 20 mg. of dye. Hence, $(20 \div 5) \times 100 = 400$ is the dye number of the sugar.

If the moisture content of the sugar does not exceed 2 per cent no correction need be applied to the dye number, but if it does, the dye number should be based on the sugar solids.

It is always advisable to wash each sample of raw sugar in a centrifugal to 99 purity, and to determine the dye number of the washed sugar also. This will give an idea of the colloids contained in the crystal and in the molasses film.

General Dye Test Procedure, Applicable to All Factory Products. The dye number of mixed, clarified, or filtered juice may be determined at the original density. In the case of sirups, massecuites, molasses, and also sugars, the sample used for the Brix determination may be conveniently diluted to 100 ml. The size of the aliquot taken depends upon the character of the product examined and may be determined by a preliminary test. The proper dilution is chosen so that the quantity of dye solution required for 100 ml. lies as much as possible between 15 and 25 ml. After adjusting the *p*H to the desired value, the test is made, and the dye number calculated as described above, on the basis of grams solids in 100 ml. of the solution of the sugar product. The operations should be standardized closely with regard to time consumed in mixing and reading, in order to obtain comparable results. The cataphoresis cell should be cleaned every few days with alcohol or hydrochloric acid, and thoroughly washed with distilled water.

The work of Badollet and Paine has been criticized by Mattson,[170] who contends that the dye number is not directly proportional to the quantity of colloids present. But this does not detract from the value of the method for comparative purposes.

Determination of the Isoelectric Point of Starch-Conversion Liquors. Paine and Badollet[171] found that the colloids in starch-converter liq-

[170] *J. Phys. Chem.*, **32**, 1532 (1928).
[171] *Facts About Sugar*, **21**, 1212 (1926); *Ind. Eng. Chem.*, **19**, 1245 (1927).

uors carry a positive electric charge, probably owing to the high acidity of the medium. The isoelectric point, at which maximum flocculation occurs, is in this case determined by adding increasing quantities of sodium carbonate solution to a measured quantity of converter liquor and making observations in the cataphoresis cell, as previously described, until migration ceases. The *p*H of the solution at the isoelectric point is the optimum for clarification of the liquor. The isoelectric point may also be determined by addition of a negatively charged colloid, such as bentonite.

Colloids and Surface Tension. Methods for the determination of surface tension have been described in Chapter XII, pp. 539–548, and it has been shown that reliable conclusions can be based only on static values. In the determination of the static surface tension of sugar products containing colloids it is not good practice to repeat measurements by the ring method on the same solution until a constant figure is obtained, because every time the ring breaks off a certain amount of agitation ensues and delays the attainment of equilibrium. Contamination of the solution is also difficult to avoid. It is therefore better to divide the sample among a number of vessels which are kept covered until use. Measurements are made at definite time intervals, each with another solution. When two consecutive results agree with each other, the result represents the static surface tension. If the time at which equilibrium is reached is definitely known, one solution kept for that length of time will suffice.

Lindfors[172] was the first to call attention to the fact that colloids in sugar products depress the surface tension of the latter, and that conversely the surface tension is a proximate measure of the colloids. A number of investigators have since studied this subject[173] but all the older measurements were made by the dynamic method, at varying time intervals. The results are therefore not comparable with each other and have led to conflicting conclusions.

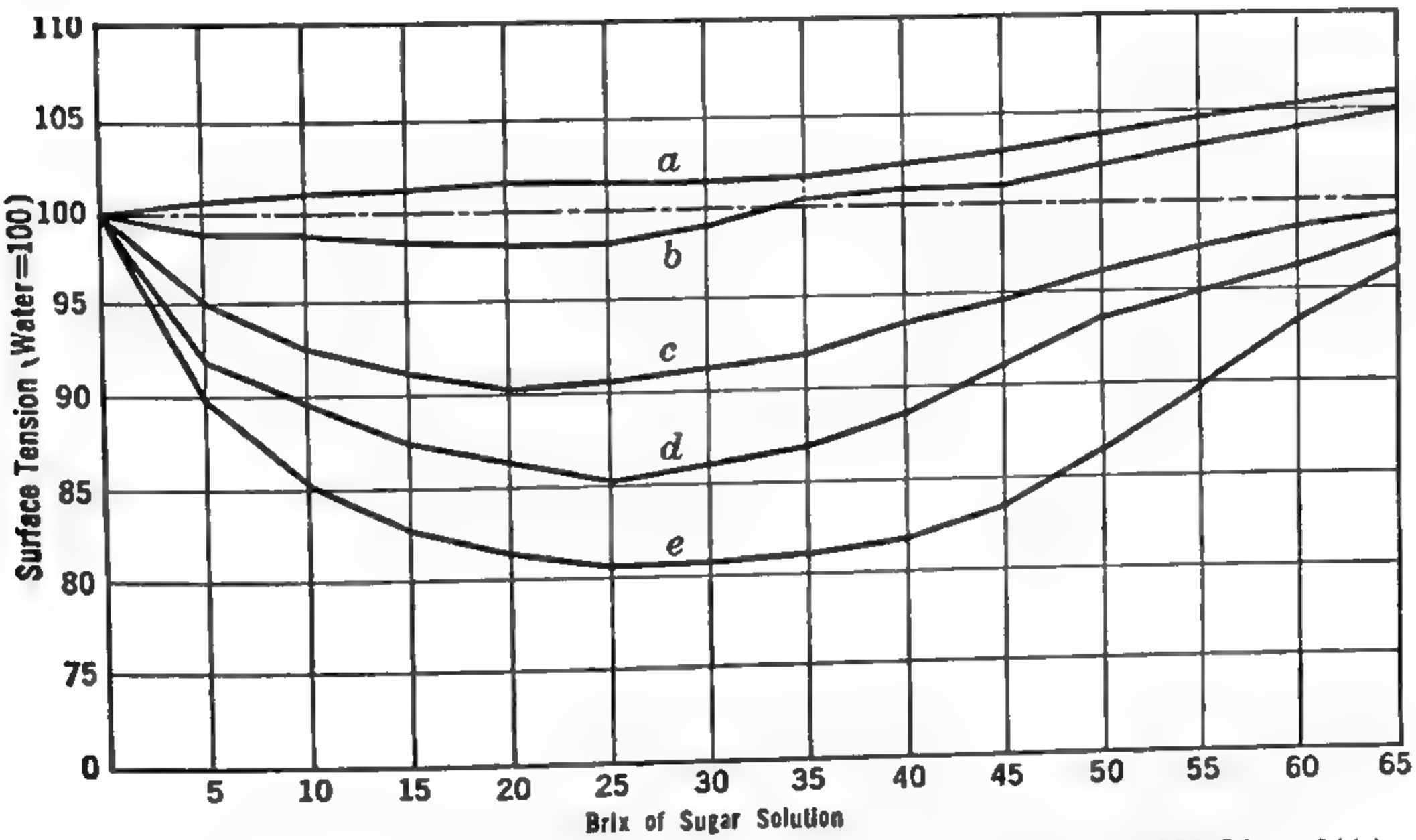
Later determinations of the static surface tension with the Du Noüy precision tensiometer, by Szymański,[174] have definitely shown not only that, as the purity falls and the colloids increase, there is a steady decrease in the surface tension below that of pure sucrose solutions of the

[172] *Ind. Eng. Chem.*, **16**, 813 (1924); **17**, 1155 (1925).

[173] Paine, Badollet, and Keane, *Ind. Eng. Chem.*, **16**, 1252 (1924); Honig, *Chem. Weekblad*, **23**, 265 (1926); Tödt, *Z. Ver. deut. Zucker-Ind.*, **76**, 253 (1926); Sázavský, *Z. Zuckerind. čechoslovak. Rep.*, **50**, 378, 423 (1926); Spengler and Landt, *Z. Ver. deut. Zucker-Ind.*, **77**, 429 (1927); Dawson, Keane, and Paine, *Intern. Sugar J.*, **30**, 33 (1928); **35**, 236 (1933); Šandera and Sigmund, *Z. Zuckerind. čechoslovak. Rep.*, **54**, 317 (1930).

[174] *Gaz. cukrownicza*, **64**, 573 (1929); **67**, 305 (1930).

same concentration, but also that, as the Brix increases, there is at first a decided decrease in the surface tension, which for beet sugars reaches a minimum at about 25° Brix, and then the surface tension rises again. This is illustrated in Fig. 321, where *a* and *b* are refined sugars, and *c* to *e* direct consumption sugars. Sugar *a* behaves like pure sucrose, showing a steady increase in the surface tension with increasing concentration, but *b* already exhibits the tendency toward a minimum value at around 25° Brix. For refinery molasses the minimum surface tension was found to be at about 40° Brix.



(Reproduced from Rept. Central Lab. Polish Sugar Industry, 1928–31, p. 641.)

FIG. 321. Graph showing relation between surface tension and concentration of sugar solutions.

There is a general relationship between the surface tension and the quantity of emulsoid colloids present in sugar products. Surface tension, however, is not an additive property, and therefore does not give quantitative results in the case of mixtures. Nevertheless, surface-tension measurements are a valuable tool to judge the general quality of sugars, especially of high-grade refined and consumption sugars, and the effect of mechanical or chemical purification processes. For comparative purposes all determinations should be made at the same temperature, the same Brix, and the same *p*H. Great care must be exercised in the interpretation of the results, because the merest traces of vegetable or mineral oils or other contaminating substances which readily find their way into technical factory products may lead to

entirely misleading results. For this reason surface-tension measurements are not adapted for routine factory control.

Foaming of Solutions of Sugar Products. Solutions of surface-active substances, and especially of emulsoid colloids, foam when they are shaken or when small gas bubbles are passed through them. The foaming is caused by the adsorption of the colloids on the air or gas bubbles, in the form of a film. The most stable foams are obtained from such colloids as soaps, saponins, or proteins, which are able to form tough, semi-solid films. The quantity of foam and its stability afford a simple, approximate measure of the emulsoid colloids present. The results do not necessarily parallel those of surface-tension measurements because foaming is affected by additional factors. Solutions with the same surface tension may give different amounts of foam; at concentrations below 15 Brix foaming practically stops, while the depression of the surface tension may be considerable. But for purposes of rapid orientation the foam test is very serviceable.

Foam Test of Paine, Badollet, and Keane, for Sugars.[175] In this test the gas bubbles necessary for foam formation are produced by heating. Fifty grams of sugar is dissolved in 25 ml. of water in a tall 200-ml. beaker, and the solution is gradually heated without stirring at a uniform rate in such a manner that the first boiling point is reached in 3.5 to 4 minutes, and a temperature of 117.8° C. in 8 to 9 minutes. The volume of the solution before heating is marked on the beaker, and again after heating to the other two temperatures. The maximum volume of foam at boiling and at 117.8° C. is an indication of the quantity of emulsoid colloids present and hence of the quality of the sugar.

Foam Test of Šandera and Mirčev.[176] These authors carry out the test at 20° C., because more foam is obtained at low than at high temperatures. The gas bubbles are produced by blowing air through the solution at a constant rate. The apparatus used is shown in Fig. 322. It consists of a tall 250-ml. cylinder of 3.5-cm. internal diameter, with a centimeter scale. Cylinders of slightly different diameter, between 2.5 and 4 cm., may be used, and the height of the foam for the standard diameter, $v_{3.5}$, may be calculated from the height v actually found by means of the formula

$$v_{3.5} = \frac{1.2\,v}{4.7 - d}$$

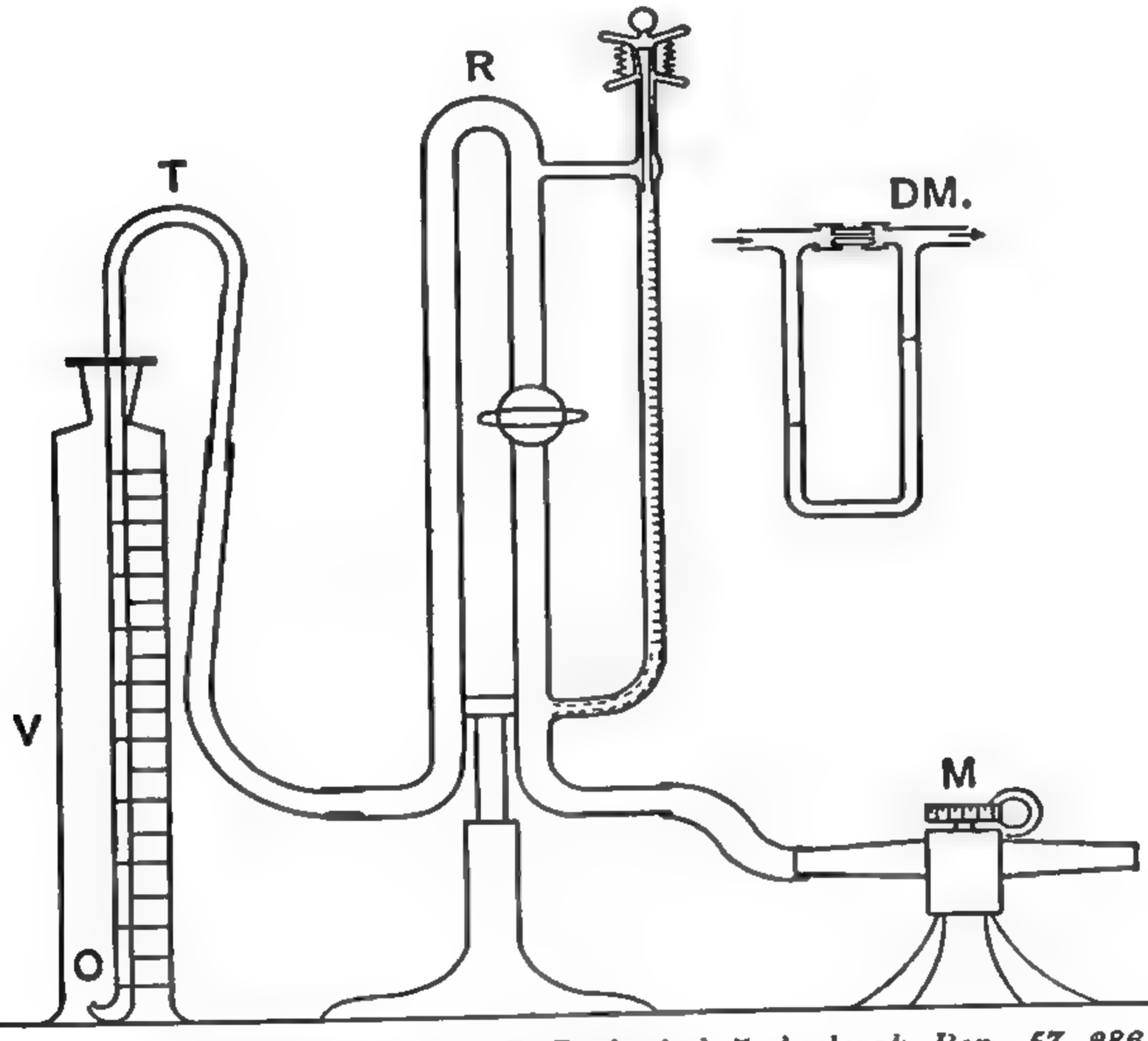
where d is the diameter of the cylinder. The air is introduced through a glass tube T which is bent at the lower end in form of a U, with a

[175] *Ind. Eng. Chem.*, **16**, 1252 (1924).

[176] *Z. Zuckerind. čechoslovak. Rep.*, **57**, 286 (1932/33).

ground tip of 1-mm. diameter. The rate of flow is regulated by micrometer valve *M*, and measured by rotameter *R*;[177] a differential manometer, *DM*, may be used instead. A velocity of 1 liter per minute is sufficient.

Fifty milliliters of the solution is measured into the cylinder, air is passed through for 1 minute, and the height of the foam generated during this time is measured in centimeters. The total time elapsed from



(Reproduced from Z. Zuckerind. Čechoslovak. Rep., 57, 286.)

FIG. 322. Apparatus of Šandera and Mirčev for the foam test.

the moment the air current is stopped until the larger bubbles have completely collapsed is also determined, in seconds. Only a ring of fine bubbles along the wall of the cylinder should remain at the end of the test.

The sugar concentration has a marked influence on the height of the foam, which increases up to about 55 Brix and falls rapidly beyond 60 Brix. The stability of the foam rises steadily up to the highest concentrations. It is therefore necessary to adopt a standard concentration, preferably lying between 30 and 55 Brix. The *p*H also has a pronounced effect, more so than on the surface tension, and all solutions to be compared should be adjusted to the same *p*H. Acid solutions of beet products evolve more foam than alkaline solutions.

[177] *Ind. Chemist*, 1, 473 (1925).

Suspended matter present in the solutions depresses the height and the stability of the foam; it may be removed by filtration through filter paper if desired.

With 50-Brix solutions of raw sugars Šandera and Mirčev found foam heights ranging from 1.5 to 23 cm., and stability figures from 10 to over 3600 seconds; molasses solutions of the same concentration gave 10 to 26 cm., and 390 to over 7200 seconds, respectively.

III. Evaluation of Refined and White Consumption Sugars

The suitability of refined and other high-grade sugars for use in candy making, jelly production, and other manufacturing processes is affected by small quantities, sometimes the merest traces, of impurities which have not been completely removed by crystallization. A number of chemical and physical methods are employed to judge the quality of these sugars. Some of these methods, as the determination of reducing sugars, ash, moisture, color, turbidity, acidity, *p*H, colloids, and grain size, have already been described. A few other methods, used more particularly for testing high-grade sugars, are added here. In some manufacturing processes the sugar is heated to high temperatures at which it undergoes a certain amount of decomposition. The degree of this decomposition, which is proportional to the amount and kind of impurities present, may be measured by the caramelization test or the barley candy test.

Caramelization Test. In this test, described by Pucherna,[178] the half-normal weight (13 g.) of the sugar is weighed into a test tube, which is immersed for exactly 15 minutes in an oil bath maintained at 170° C. The test tube is removed and allowed to cool spontaneously to room temperature. The sugar is dissolved to a total volume of 50 ml., and the color of the resulting solution is measured. Other determinations may also be made if desired, such as polarization, sucrose, reducing sugars, and surface tension.

Barley Candy Test. This test, originally devised by S. C. Hooker, follows more closely the procedure used in making hard candy, the sugar being heated in the presence of water. The method is described by Ambler and Byall as follows:[179]

The apparatus, Fig. 323, consists of a copper casserole beaten from 0.16-cm. stock, 11.6 and 5.7 cm. in diameter at top and bottom, respectively, and

[178] *Z. Zuckerind. čechoslovak. Rep.*, 55, 144, 663 (1930/31).

[179] *Ind. Eng. Chem., Anal. Ed.*, 7, 168 (1935); see also Ambler, "Methods for determining the uniformity of quality of white sugars," p. 31, 1935.

6.5 cm. deep, with a hollow handle 5 cm. long, which is made of the same stock, riveted to the side, and into which is fitted a handle of oak about 17.5 cm. long.

Two hundred and twenty-seven grams of sugar and 90 ml. of distilled water are placed in the casserole, thoroughly mixed, and heated on a Chaddock

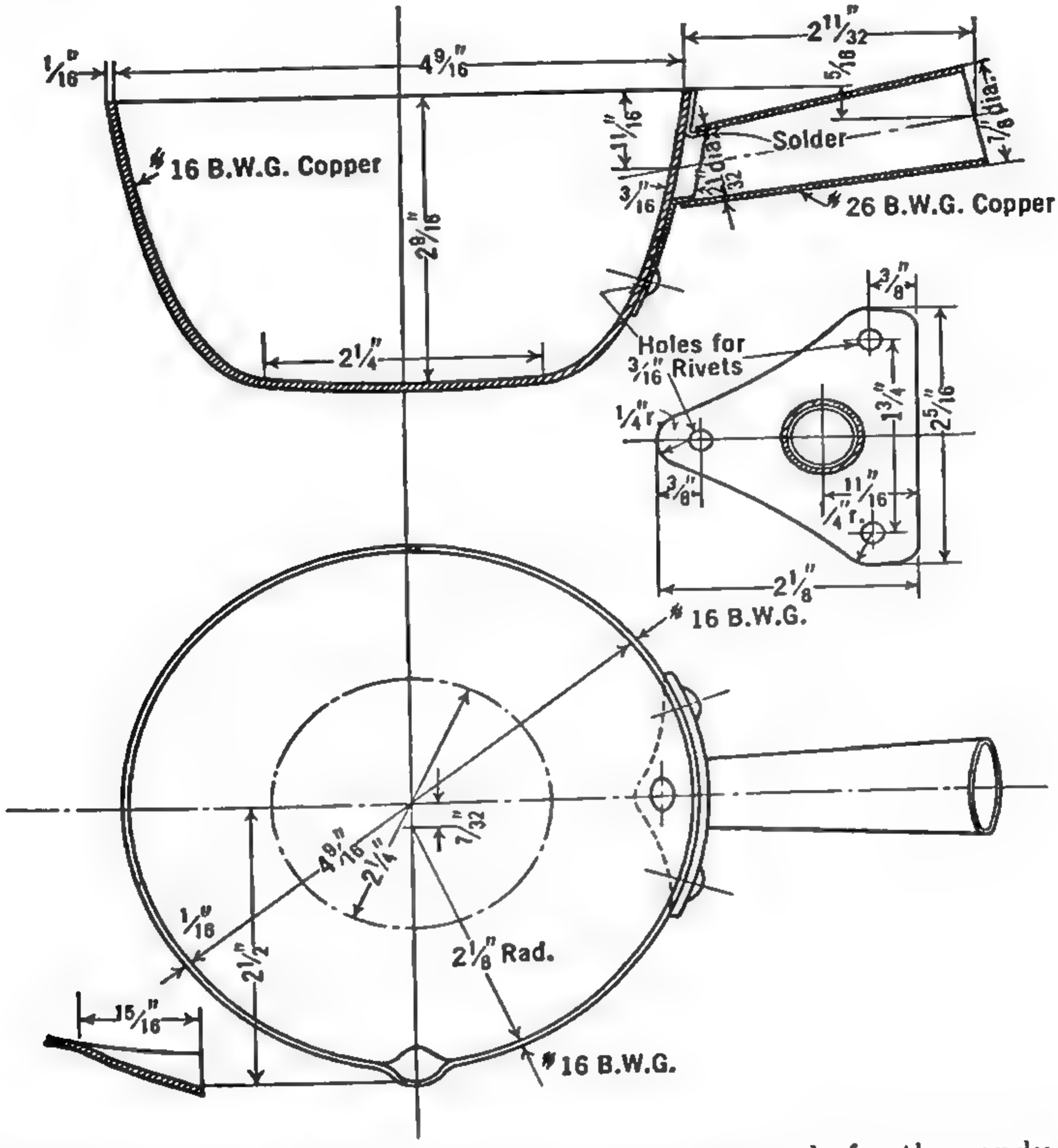


FIG. 323. Cross section and top view of copper casserole for the candy test.

burner with gas-pressure regulation in a place shielded from draughts, with constant stirring, until all the sugar has been dissolved, after which the stirring rod is removed and the sirup is left undisturbed until it commences to boil. The pressure and velocity of the gas admitted to the burner are so regulated that they are constant and bring the sirup to boiling in 5 to 5.5 minutes from the time heating was started. The casserole is then immediately covered with a 15-cm. watch glass, and the sirup is allowed to boil undisturbed. (If the sirup foams so badly on boiling that there is danger of its overflowing, the casserole is lifted momentarily from the burner to allow the foam to subside, and the watch glass is placed in position as soon as the foam

breaks and danger of overflowing has passed. When such foaming occurs it is noted as a characteristic of the sugar.)

Exactly 15 minutes after the heating was started the watch glass is removed. Heating is continued, the mixture being constantly stirred with a standard thermometer until the temperature of the boiling sirup reaches 175° C. During the entire test the pressure and velocity of the gas are kept constant. The total time of heating is between 21 and 25 minutes, and, if the gas is properly regulated, does not vary more than ±0.5 minute in the different tests of any series. As soon as the sirup has reached the temperature of 175° C. it is quickly poured out on a cold, dry cooling plate of polished copper, 35 by 35 by 0.6 cm. in dimensions. When the candy plaque so formed has cooled and hardened, it is broken up, bottled, and used as soon as possible for analytical determinations.

The procedure described must be adhered to in every detail, since slight modifications may cause large differences in the results.

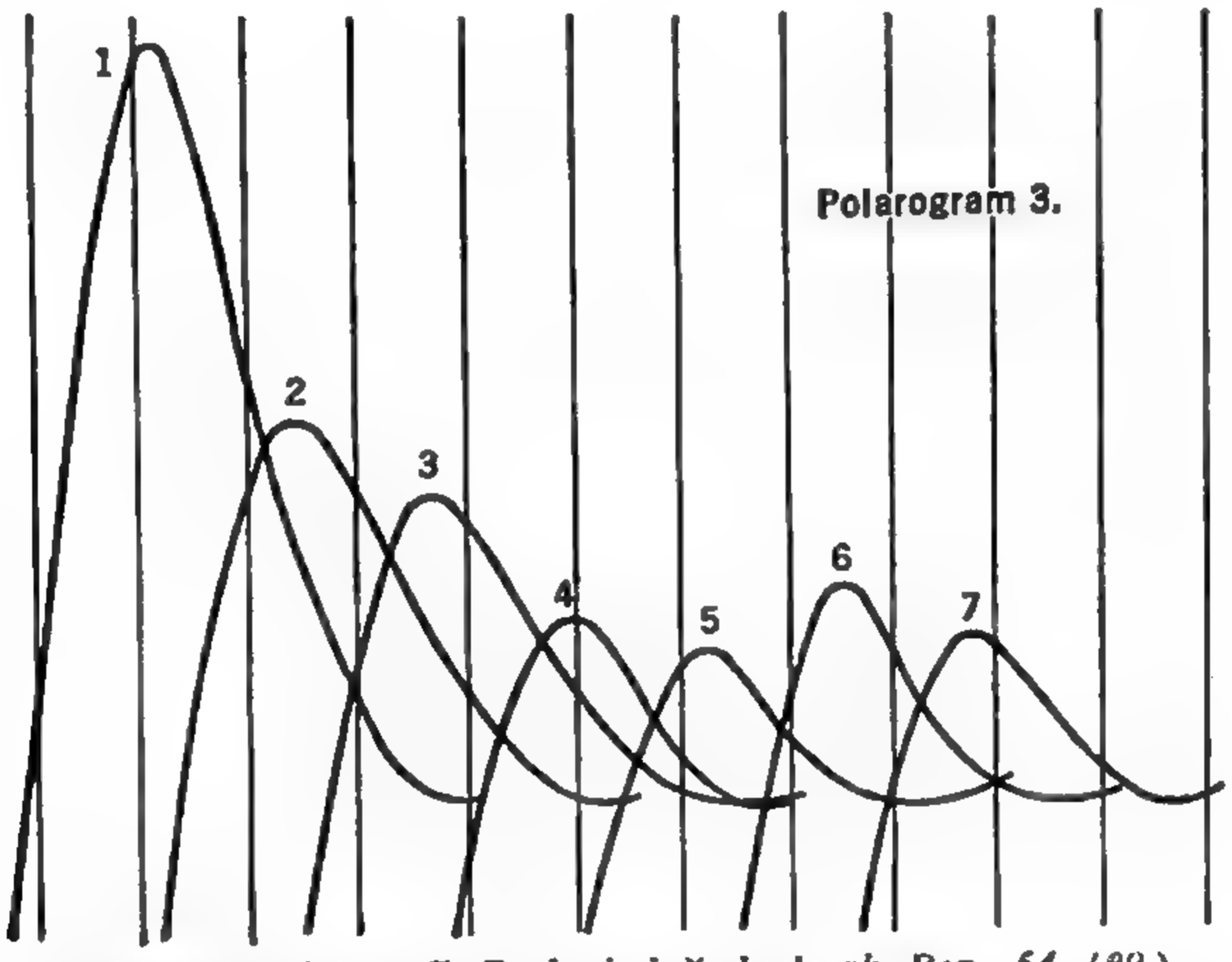
The color of the resulting candy and its content of sucrose and reducing sugars indicate the quality of the sugar. Pure sucrose and high-grade refined sugars with very low ash content undergo a certain amount of inversion, because sucrose itself has acidic properties, enhanced at high temperatures. As the ash content increases it exerts a buffering effect; there is less inversion, but the alkalinity of the ash causes greater coloration. Neutral or acid salts generally increase the amount of invert sugar formed but give light-colored candies. Alkaline salts or salts of volatile acids produce less inversion but greater destruction of invert sugar with consequent high color formation. Ammonium salts and amino acids cause strong inversion and also the formation of dark-colored, nitrogenous substances. Iron salts are in a class by themselves, producing strong inversion owing to hydrolysis, and also very dark-colored polyhydroxy compounds of iron.

The barley candy test may also be used to judge the quality of commercial glucose sirup added to a standard cane sugar, or of commercial dextrose.

Polarographic Method for Judging the Quality of White Sugars. Traces of surface-active substances present in refined sugars may be determined by the polarographic method, which has been developed mainly by Heyrovský and his school and applied to sugar analysis by Šandera and Zimmermann.[180] A small quantity of an electrolyte is added to the sugar solution, and the latter is electrolyzed in the presence of air. The solution is placed in a beaker or flask on a layer of mercury which serves as the anode, the cathode being formed by drops of mercury that fall into the solution through a capillary the lower end

[180] *Z. Zuckerind. čechoslovak. Rep.*, **53, 383 (1928/29); 54, 394, 425 (1929/30).**

of which is immersed in the sugar solution. The cathodic potential is slowly increased by means of a sliding contact which is connected with the dropping mercury cathode through a sensitive galvanometer. The galvanometer deflection increases with the rise in potential until the point is reached where all the oxygen adsorbed on the dropping cathode is reduced. At that moment a thin, exhausted layer forms between the cathode surface and the solution. After this the deflection of the galvanometer is determined only by the velocity with which the oxygen diffuses to the protecting layer; it therefore decreases rapidly, and



(Reproduced from Z. Zuckerind. Czechoslovak. Rep., 54, 429.)

FIG. 324. Polarogram of coloring matters in beet molasses.

1: 0.002 *M* potassium sulfate.
2: Same, plus 5.2 per cent sucrose.
3: Same as 2, plus 0.0002 per cent ulmin.
4: Same as 2, plus 0.0002 per cent caramel.
5: Same as 2, plus 0.0002 per cent coloring matter from molasses I.
6: Same as 2, plus 0.0002 per cent coloring matter from molasses II.
7: Same as 2, plus 0.0002 per cent molasses colloids.

finally becomes practically constant. If any surface-active substances, as coloring matter or other colloids, are present in the sugar solution they displace the oxygen adsorbed on the cathode, and the maximum galvanometer deflection becomes much smaller. Heyrovský and Shikata[181] have designed an apparatus in which the galvanometer deflection is registered automatically on a drum covered with light-sensitive paper so that a complete graphic record is obtained during the electrolysis, which requires only about 20 minutes. Several such "polarographic" curves, obtained by Zimmermann, are shown in Fig. 324. A 5.2 per

[181] *Rec. trav. chim.*, 46, 496 (1925).

cent solution of purified sucrose in 0.002 *M* potassium sulfate solution was electrolyzed, alone, and after the addition of 0.0002 per cent of various coloring matters or colloids. The depressing effect of these substances on the oxygen maximum is clearly apparent.

Polarographic curves may also be used for detecting incipient inversion of white sugars.[182] In this case all the dissolved air in the neutral or slightly alkaline solution is expelled by passing hydrogen through it. Under this condition ketoses are reduced, while aldoses and sucrose are not affected. Reduction takes place at a definite potential, and the current intensity increases in direct proportion to the concentration of the ketose.

A similar procedure has been used by Cantor and Peniston[183] to determine the proportion of the aldehydo- (straight-chain) modification in a number of aldoses (see p. 641), by comparing the voltage curves with those of known aldehydes.

Examination of Sugars with Ultraviolet Radiation. Lundén[184] found that the quality of white sugars may be judged by the fluorescence which ultraviolet radiation produces in them. A mercury-vapor lamp, with a uviol filter which absorbs the visible rays, is employed for the purpose. Pure sucrose or a very high-grade refined sugar is used as a standard. The two sugars to be compared are dissolved in water to the same concentration (between 40 and 60 Brix), and the solutions filled into cells of non-fluorescent glass, open on top. The two cells are placed below the lamp, side by side, so that the surfaces of the two solutions are always in the same plane. The portions directly below the surface of the solutions are observed through two holes in a cardboard screen, light filters being held in front of the eye. High-grade refined sugars usually show only small differences at the blue end of the fluorescence spectrum, and the best results are obtained with an orange-red filter (590 to 700 mμ). Roughly quantitative measurements are made by diluting the solution of the sample until the intensity equals that of the standard. If the solution of the sample has to be diluted to twice its original volume in order to match the standard, the fluorescence intensity of the sample is twice that of the standard. The results obtained by Lundén with three white beet sugars are given in the table below. The standard sugar was a very high-grade refined, containing 0.001 per cent ash; the second was a lower-grade refined, with 0.01 per

[182] Heyrovský and Smoleř, *Chem. Listy*, **26**, 479 (1932); Sidersky, *Bull. assoc. chim. sucr. dist.*, **50**, 400 (1933).

[183] *J. Am. Chem. Soc.*, **62**, 2113 (1940).

[184] *Centr. Zuckerind.*, **33**, 1281 (1925); **35**, 219 (1927); *Z. Zuckerind. čechoslovak. Rep.*, **51**, 304 (1926/27).

cent ash; and the third a white consumption sugar, with 0.03 per cent ash. The figures shown are the relative fluorescence intensities.

	Blue Filter 448–473 mμ	Red Filter 590–700 mμ
No. 1 (Standard)....	1	1
No. 2.....	1.2	1.9
No. 3.....	2.4	2.7

Cane sugars gave similar results, the fluorescence in the red increasing with decreasing quality. The tests may also be made with the solid sugars, but only if the crystals are of approximately the same size. The presence of bluing agents does not affect the results.

Very little is known about the substance or substances which cause the fluorescence. Sucrose itself, in solid form or in solution, gives a faint blue fluorescence. According to Šandera,[185] caramel and other deeply colored impurities are not responsible for the fluorescence, which is due to a colorless or slightly colored substance, soluble in ether or chloroform, and probably an intermediate product of the effect of alkali on reducing sugars.

The fluorescence of sugar products in ultraviolet light may be utilized for a variety of other purposes. Gérard[186] has reported that diluted cane molasses, soaked into filter paper, produces a brown or yellow fluorescence, while beet molasses under the same conditions gives a blue or greenish light. These and similar statements, however, require further study and confirmation.

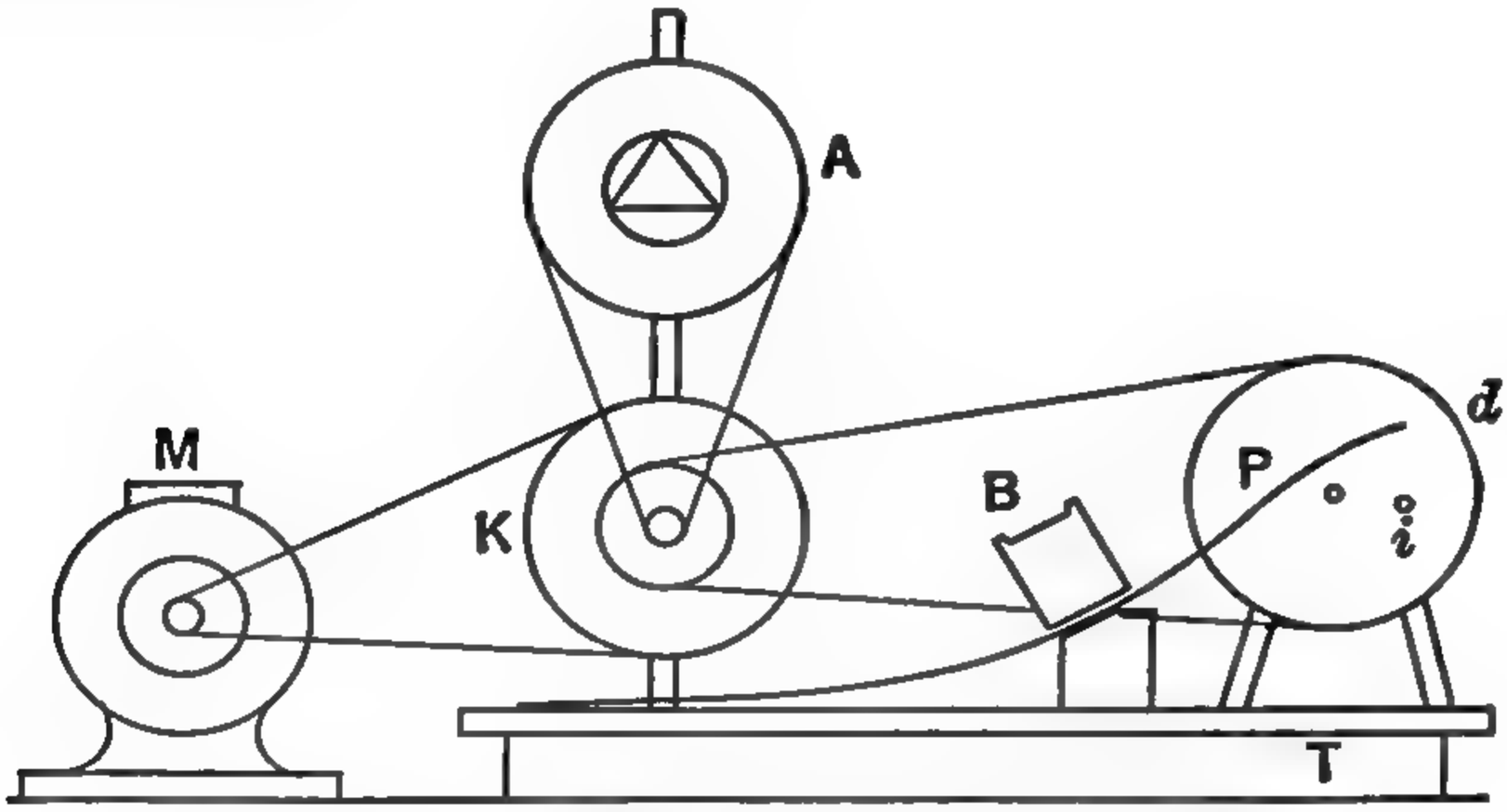
SPECIAL PHYSICAL TESTS FOR MOLDED AND PRESSED SUGARS

Refined sugars in the form of tablets, cubes, loaves, and pilé, are subject to damage during transportation and handling, and various tests have been devised to determine their breaking strength or "firmness." This quality is also related to the rapidity with which these sugars disintegrate when dissolved in water. Some of the methods used for measuring these properties are described here.

Determination of Resistance to Abrasion. The breaking strength of these sugars may be determined with machines of the type used in the testing of cement and similar materials. Simpler methods have been developed by Šandera and Zimmermann.[187] The apparatus used by these authors is shown in Fig. 325. *M* is a small electric motor. This is connected through transmission *K* with a disk *A* to which a sam-

[185] *Z. Zuckerind. čechoslovak. Rep.*, 51, 237, 323 (1926/27).
[186] *Ann. fals.*, 25, 212 (1932).
[187] *Z. Zuckerind. čechoslovak. Rep.*, 56, 481 (1931/32).

ple can, 8 cm. in diameter by 9 cm. high, is fastened horizontally. The transmission is adjusted so that the can makes 150 r.p.m. around its axis. Another transmission connects *K* with the disk *d*. An elastic metal band *P* is mounted at one end on the base board, while the free end, at each revolution of *d*, is alternately lifted by pin *i* and released again. Disk *d* makes 220 r.p.m. A second sample tin *B* is soldered to the elastic band as shown in the sketch, with a buffer block placed below the band at this point.



(Reproduced from Z. Zuckerind. Čechoslovak. Rep., 56, 482.)

FIG. 325. Apparatus for determining resistance of lump sugar to abrasion.

One hundred and fifty grams of the sugar to be tested is weighed into each of the tins, and the motor is run for 30 minutes. The sugar is then removed and sifted with a coarse sieve, and the sugar remaining on the sieve is reweighed. The result, expressed as per cent of the original weight, is a measure of the resistance to abrasion by revolving and by shock, respectively. At least three determinations are made, and the results are averaged. The discrepancies among individual determinations are usually within 2 per cent. Sugars are more resistant to intermittent shocks than to abrasion by continuous motion.

Determination of the Speed of Solution. The time required to dissolve completely a given quantity of sugar is important for consumers in industry as well as in the home. Koydl[188] made a study of this subject, using a small cylindrical vessel of metal, the bottom of which was formed of finest silk gauze. By means of a mechanical arrangement, the vessel was alternately lowered into and raised above the surface of water contained in a small tank beneath, at the rate of 60 dippings per minute. Under these conditions 5 g. of various types of powdered, crystal, pressed, and molded sugars required from 0.3 to 8.5 minutes for complete solution.

[188] *Z. Zuckerind. Böhmen,* **34, 445 (1909/10).**

According to Šandera,[189] the speed of solution R, as a first approximation, is directly proportional to the weight m of the sugar and indirectly proportional to its surface p and the time t, or

$$R = \frac{m}{pt} \text{ grams per cm.}^2 \text{ surface per second} \qquad (1)$$

The term p may be eliminated from the equation by expressing m and p as functions of the radius r of the crystals and of the specific gravity S (1.58 for sugar):

$$m = k_1 r^3 S \qquad (2)$$

$$p = k_2 r^2 \qquad (3)$$

Then $$p = \frac{k_2}{k_1^{2/3} \times S^{2/3}} \times m^{2/3} = k m^{2/3}$$

where k, k_1, and k_2 are constants.

Substituting this value of p in equation (1), we obtain

$$R = \frac{K \sqrt[3]{m}}{t}$$

where

$$K = \frac{v^{2/3} S^{2/3}}{p},$$

v being the volume and K a new constant. For a spherical particle of sugar K equals 0.28; for a cube, 0.22; for an average normal sugar crystal, 0.203, etc. By means of the equations given above, Šandera determined R with measured crystals, and with spheres of amorphous sugar produced by rapid cooling of a highly concentrated sugar solution. For practical purposes it is not necessary to measure R in absolute units, and the operations necessary can be greatly simplified.

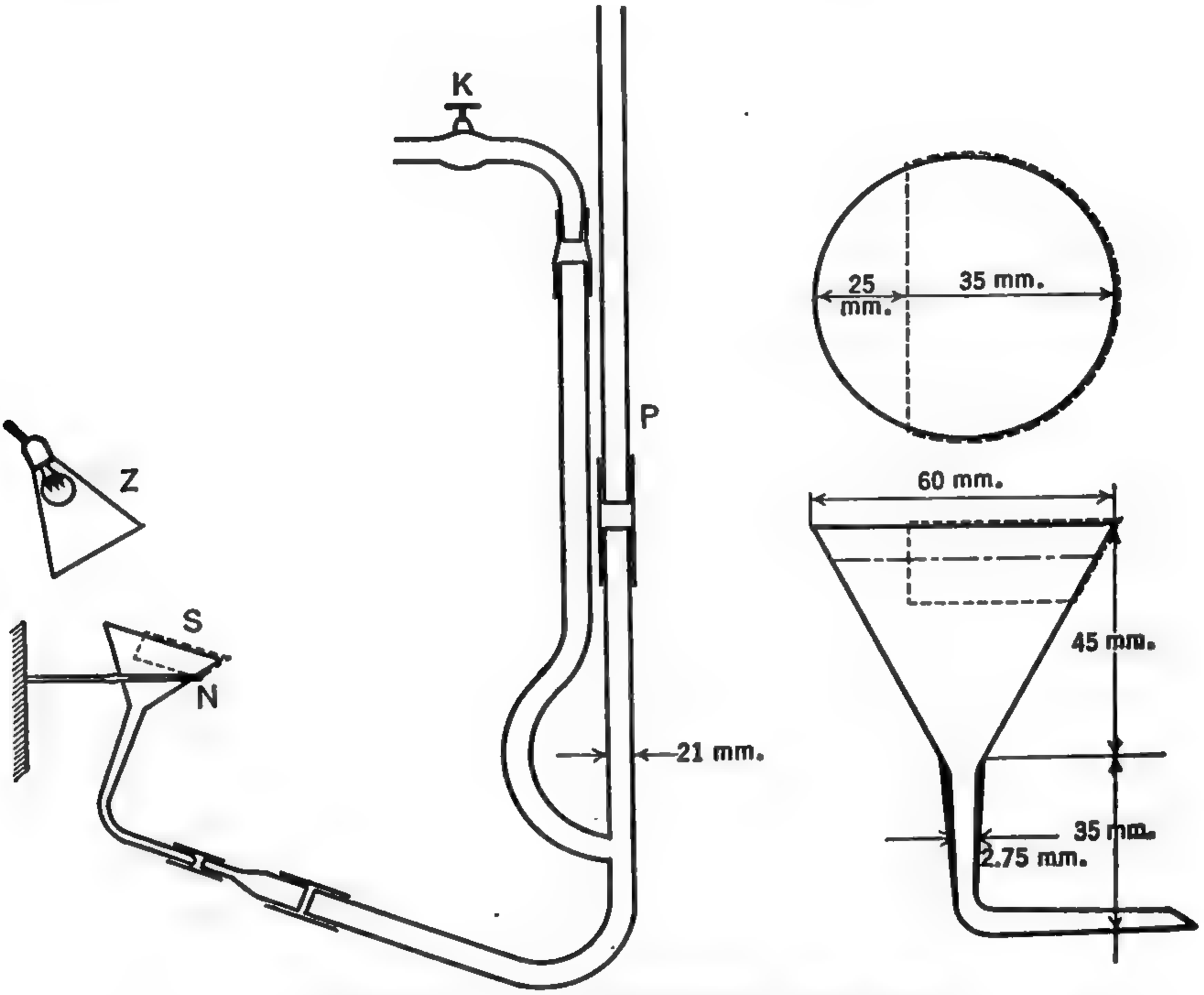
For this purpose Šandera and Mirčev[190] devised the apparatus shown in Fig. 326. It consists of a glass tube attached to a water tap K, and connected with an overflow tube P to regulate the speed of the water flow. The lower end of the tube is connected with a funnel N,[191] placed in an inclined position and provided with a sieve S, to keep the sugar from floating away after the water has been turned on. The sieve must be kept clean by frequent washing with dilute ammonia. The apparatus is filled with water, the water turned off, and a piece of the pressed or molded sugar, weighing between 2 and 10 g., is placed on the sieve in

[189] *Z. Zuckerind. čechoslovak. Rep.*, 52, 153 (1927/28).

[190] *Z. Zuckerind. čechoslovak. Rep.*, 57, 217 (1932/33).

[191] Standard funnels may be obtained from the Research Institute for the Czechoslovakian Sugar Industry, Prague.

the funnel. It is left undisturbed for 1 minute, and then the water is turned on and the flow adjusted so that 800 ml. passes per minute. The sugar is closely observed during this process with the aid of the small lamp *Z*. The time from the moment when the sugar is introduced until it is completely dissolved is measured with a stop watch.



(*Reproduced from Z. Zuckerind. Čechoslovak. Rep., 57, 218.*)

FIG. 326. Apparatus for determining speed of solution of lump sugar.

The results are expressed not in terms of R, but of its reciprocal D, which is the observed time t, multiplied by appropriate factors k_m and k_t, to reduce it to the standard weight of 5 g. sugar and to the standard temperature of 20° C.:

$$D_5^{20} = tk_mk_t$$

Since R is directly proportional to m

$$D_5 = \frac{t\sqrt[3]{5}}{\sqrt[3]{m}}$$

hence

$$k_m = \sqrt[3]{\frac{5}{m}}$$

The values of k_m for varying weights of sugar between 2 and 10 g. are shown in Table CXXXVI.

The temperature effect was measured by Netuka,[192] later again by Šandera and Mirčev,[193] and the following simple relationship was found as the average of a large number of determinations:

$$k_t = 0.05\,t$$

where t signifies degrees C. Thus for 20° C., the normal temperature, $k_t = 1$, at 16° C. = 0.80, etc.

TABLE CXXXVI

VALUES OF THE WEIGHT CORRECTIONS

m	k_m	m	k_m	m	k_m	m	k_m
grams		grams		grams		grams	
2.0	1.36	4.0	1.08	6.0	0.94	8.0	0.85
2.2	1.31	4.2	1.06	6.2	0.93	8.2	0.85
2.4	1.28	4.4	1.04	6.4	0.92	8.4	0.84
2.6	1.24	4.6	1.03	6.6	0.91	8.6	0.83
2.8	1.21	4.8	1.01	6.8	0.90	8.8	0.83
3.0	1.19	5.0	1.00	7.0	0.89	9.0	0.82
3.2	1.16	5.2	0.99	7.2	0.88	9.2	0.82
3.4	1.14	5.4	0.98	7.4	0.88	9.4	0.81
3.6	1.11	5.6	0.96	7.6	0.87	9.6	0.80
3.8	1.10	5.8	0.95	7.8	0.86	9.8	0.80
...	....	...	....	...	....	10.0	0.79

Example. A piece of loaf sugar weighing 6.6 g. required 6 minutes and 35 seconds to dissolve completely in water of 18° C. Hence D_5^{20} equals 395 × 0.91 × 0.90 = 323.5 seconds.

This method is used in Czechoslovakia, and has given satisfactory results.[194] The water should be free from suspended matter, but dissolved salts do not interfere.

The previous history of the sugar has an important effect on the speed of solution.[195] Sugar that has been heated to a high temperature dissolves more rapidly. Sugar that has taken up moisture usually dissolves more slowly, but there are exceptions to this rule, depending on the type of cube and on the conditions under which the sugar has become moist. Drying at room temperature has no effect.

[192] *Z. Zuckerind. čechoslovak. Rep.*, **52**, 289 (1927/28).
[193] *Z. Zuckerind. čechoslovak. Rep.*, **60**, 233 (1935/36).
[194] Dolínek, *Z. Zuckerind. čechoslovak. Rep.*, **58**, 363 (1933/34).
[195] Šandera and Mirčev, *Z. Zuckerind. čechoslovak. Rep.*, **61**, 25 (1936/37).

The Break-Down Test. In practice it is frequently not necessary to resort to the tedious procedure just described. For comparative purposes it is sufficient to observe the time required for the agglomerated sugar to disintegrate, without dissolving completely. In some American refineries this so-called " break-down " test is carried out by immersing several cubes or tablets in water at 20° C. on a 4-mesh screen (4.76-mm. openings). The average time required for the pieces to pass completely through the screen is recorded.

This method has been studied by Schäffer,[196] Kořán,[197] Šandera and Mirčev,[198] and others, and the following two procedures have been developed by Šandera:[199]

Sieve Method of Šandera. The flat sieve employed in this method is 15 by 14 cm., made of brass wire 1 mm. thick, with openings 6.7 mm. square (13 openings for 100 mm.), and soldered at the edges to make it more rigid. It is suspended by wires from a stand. Ten sugar tablets are placed, resting on their long, narrow edges, upon the dry sieve which is then lowered into a large glass jar filled with water at 20° C., so that the sieve is 2.5 cm. below the surface of the water, and 15 cm. above the bottom of the jar, and the time is noted with a stop watch. The time, in seconds, after which the sixth tablet passes completely through the screen is recorded, experiments having shown that this agrees, within the limit of error of the method, with the average time for the disintegration of the ten tablets.

Float Method of Šandera. In this method a sieve of tinned wire of 0.4-mm. gauge, with openings 1 by 1 mm., is used. The sieve, 9 cm. wide, is bent in the form of an inverted W. Three sugar tablets each are placed flat on the outside inclined surfaces, and two each on the inside surfaces. The sieve is attached rigidly by stout wire to a float placed above it and made conveniently of a discarded electric-light bulb. The float is suspended from a stand by a piece of thread and is tared so that it will rise in water when 90 to 95 per cent of the sugar has passed through the sieve. The apparatus is immersed in water at 20° C., and the time is noted when the float begins to rise and the thread loosens. For comparative purposes it is necessary to reduce the results to a standard weight of sugar, and if the temperature of the water differs from 20° C. a correction must be applied. These calculations are made by the formula given on p. 1116.

The float method gives more exact results than the sieve method,

[196] *Deut. Zuckerind.*, **57**, 630 (1932).
[197] *Z. Zuckerind. čechoslovak. Rep.*, **58**, 316 (1933/34).
[198] *Z. Zuckerind. čechoslovak. Rep.*, **60**, 233, 241 (1935/36).
[199] *Z. Zuckerind. čechoslovak. Rep.*, **61**, 276 (1936/37); **62**, 153 (1937/38).

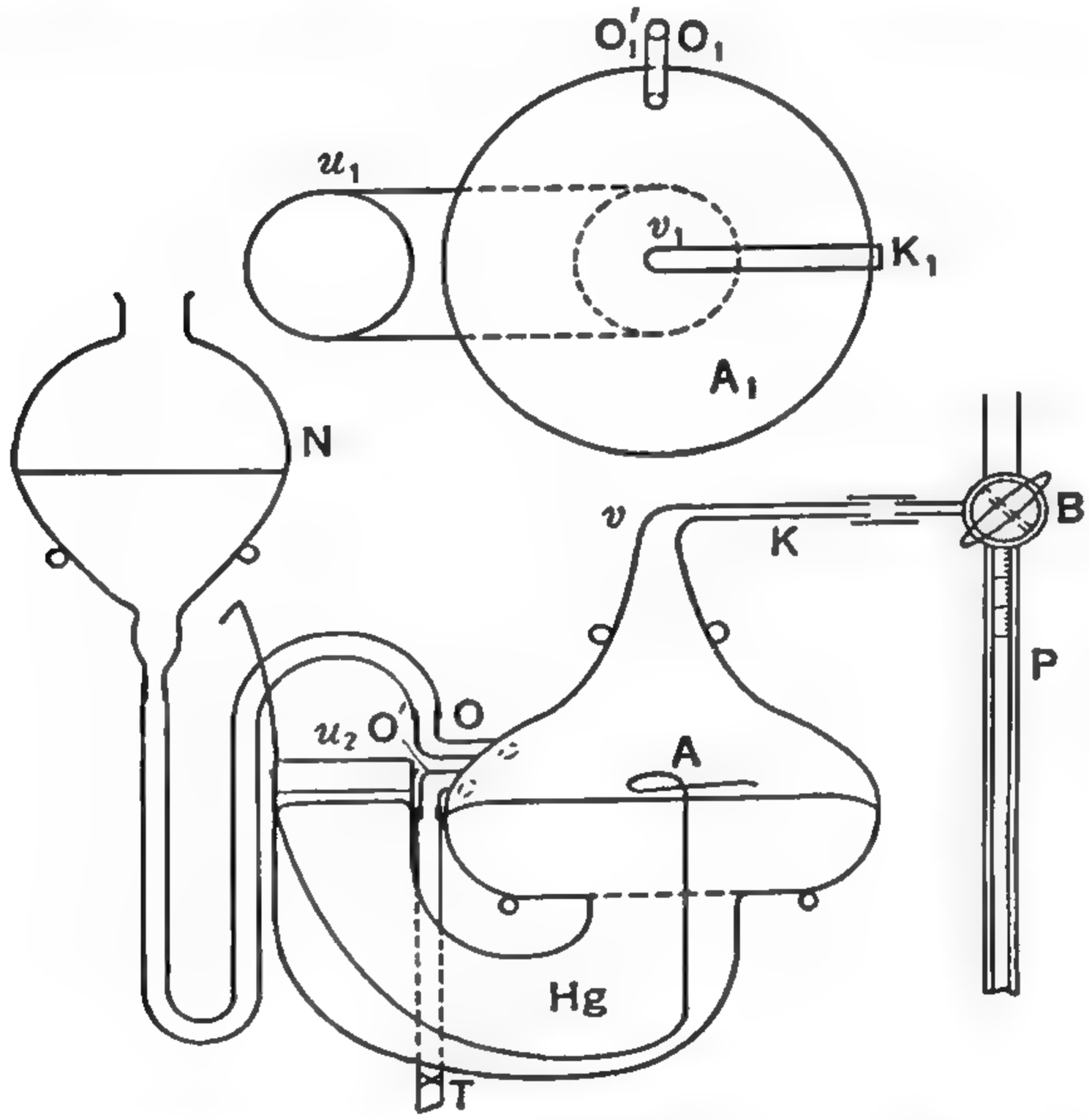
but the latter is simpler and sufficiently accurate for control purposes.

Determination of Apparent Specific Gravity. This property is a measure of the air-filled pores in molded or pressed sugars, and thus indirectly of the firmness, but the results obtained do not necessarily agree with the resistance to abrasion, because the methods used are fundamentally different.

Method of Staněk and Šandera.[200] In this method the volume v of air occluded in a piece of sugar weighing m grams is measured, and the apparent specific gravity S calculated by the formula

$$S = \frac{m}{m/1.58 + v} = \frac{1.58\,m}{m + 1.58\,v}$$

where 1.58 is the specific gravity of sucrose. The sugar is dissolved in water, and the liberated air is measured. It is impossible, however,



(Reproduced from Z. Zuckerind. Čechoslovak. Rep., 56. 396.)

FIG. 327. Apparatus of Staněk and Šandera for measuring the apparent specific gravity of lump sugar.

to immerse the sugar directly in water without introducing adhering air bubbles, or without some of the air in the surface pores escaping unmeasured. The sugar is therefore introduced into the water through mercury. The apparatus used is shown in Fig. 327. It consists of the

[200] *Z. Zuckerind. čechoslovak. Rep.*, 56, 396 (1931/32).

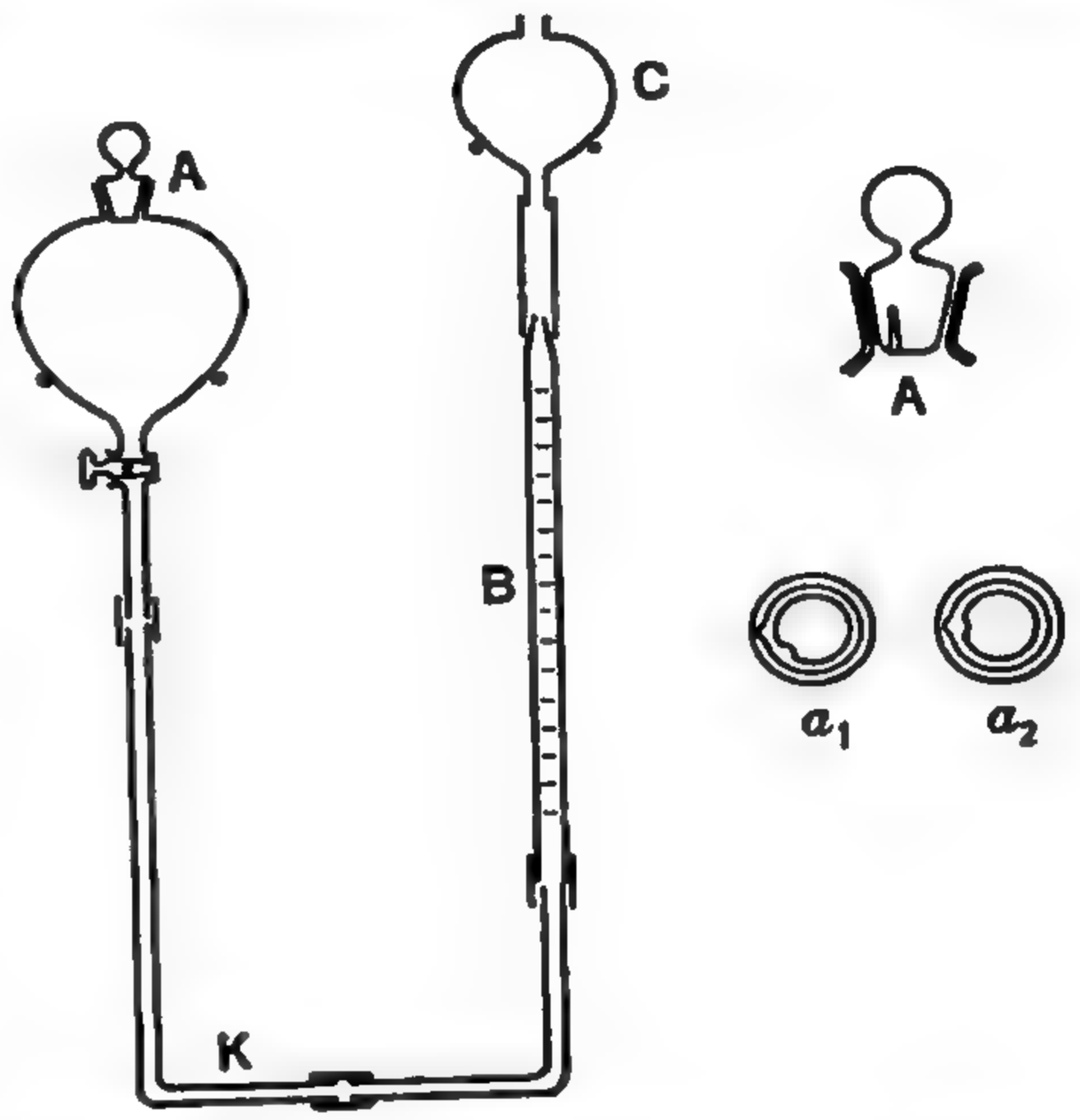
solution vessel, *A*, connected at the bottom with a wide U-shaped tube which is closed by the stopper u_2. The vessel is filled with mercury up to the level shown in the sketch. The top of the vessel *A* is connected through tube *K* and two-way stopcock *B* with the measuring burette *P*, the lower end of which is immersed in water. In the rear of *A* (correctly shown in the top view, but in the cross section at the left) are two outlets *O* and *O'*. *O* is connected by a rubber tube with the leveling bulb *N*; *O'* ends in a piece of rubber tubing closed by a screw clamp *T*.

The apparatus is thoroughly cleaned before use, to remove all traces of fat. The spaces above the mercury in *A*, tube *K*, and burette *P* are completely filled with water, so that no air bubbles remain. Stopper u_2 is removed, and the weighed piece of sugar is introduced into the mercury in the side arm by means of a pair of steel forceps. It is turned around several times to remove adhering air bubbles and to establish direct contact with the mercury over its entire surface. It is then pushed beyond the bend and allowed to rise to the surface of the mercury in vessel *A*. The air begins to escape at once, and collects in the top of the vessel. The sugar is completely dissolved by stirring with a piece of wire shown in the diagram. The collected air is then driven over into burette *P*, and its volume is read after adjusting the level of the water in bulb *N* with that of the water in the burette. The air in the burette is allowed to escape by opening stopcock *B*, and the apparatus is ready for another determination. When after several determinations the solution in vessel *A* becomes too concentrated, it is run out through *O'*, and replaced with fresh water. The whole apparatus must be cleaned from time to time to remove accumulated fat which causes the air bubbles to adhere to the walls. The burette should be calibrated so that it reads to ± 0.02 ml. If four or five determinations with the same sugar are averaged, the error of the mean is ± 0.01 in terms of specific gravity. The apparent specific gravity of molded or pressed sugar ranges from about 0.9 to 1.3.

Method of Šandera and Zimmermann. An apparatus for directly measuring the apparent specific gravity of molded or pressed sugars has been described by Stolle[201] and has been simplified by Šandera and Zimmermann.[202] In Fig. 328, *A* is a small separatory funnel of about 200-ml. capacity, having a wide mouth and a specially ground stopper, with channels like those used in dropping bottles (a_1: closed, a_2: open). The lower end of the funnel is connected, by means of a cemented steel capillary, with the micropipette *B*, calibrated in 1/100 ml.,

[201] *Deut. Zuckerind.*, **32, 559 (1907).**
[202] ***Z. Zuckerind. čechoslovak. Rep.*, 56, 481 (1931/32).**

and with a leveling bulb *C*. The apparatus is filled with mercury, and *C* is raised until the mercury reaches the center of the channel in the stopper on *A*. The stopper is turned to close the channel, and the pipette is read at this level. The stopper is removed, *C* lowered, and several weighed pieces of sugar are placed on top of the mercury through the neck of the separatory funnel. The stopper is replaced, with the channel open, and the mercury in *A* is slowly raised, with constant tapping to remove air bubbles, to the same point as previously. The stopper is closed, the level adjusted, and the pipette read again. The difference between the two readings gives the volume of the mercury displaced by the sugar, and this is divided into its weight, to calculate the apparent specific gravity. The entire operation is repeated several times, and the average is taken. This method is not as exact as that of Staněk and Šandera, but is well adapted for routine work because of its simplicity and rapidity.



(Reproduced from Z. Zuckerind. Čechoslovak. Rep., 56, 483.)

FIG. 328. Apparatus of Šandera and Zimmermann for measuring the apparent specific gravity of lump sugar.

BACTERIOLOGICAL EXAMINATION OF WHITE SUGARS

In 1926, E. J. Cameron, of the Research Laboratories of the National Canners' Association, was able to trace spoilage of canned peas, corn, and other non-acid products to thermophilic bacteria occurring in refined sugars. Further investigation showed that there are several types of such bacteria, that some of them are usually more prevalent in cane sugars and others in beet sugars. Raw sugars were also found to be contaminated, and the bacteria could be followed throughout the manufacturing or refining processes. A few years later the National Canners' Association established definite standards for sugars to be used for canning purposes. These standards have been revised from time to time. The following are the methods and standards adopted by the Association in 1935, and amended in 1937:

Sampling. One-half pound samples will be taken from each of five bags or barrels of the shipment or of the lot in question. These samples will be sent to the laboratory in clean sealed cans, or other appropriate containers.[203]

Preparation of Sample. Place 20 g. of sugar in a sterile 150-ml. Erlenmeyer flask marked to indicate a volume of 100 ml. Add sterile water to the 100-ml. mark. Bring rapidly to boiling, and boil for 5 minutes. Replace evaporation with sterile water.

Detection of Flat Sour Spores. Into each of five Petri dishes pipette 2 ml. of the boiled sugar solution. Cover, and mix the inoculum with Bacto-dextrose Tryptone Agar.[204] Incubate the plates at 55° C. for 36 to 48 hours. In order to prevent drying of the agar, the incubator should be humidified. The combined count from the five plates represents the number of spores in 2 g. of the original sugar. Multiply this count by 5 in order to express results in terms of number of spores per 10 g. of sugar.

Flat sour colonies are characteristic. The colony is round, measures from 2 to 5 mm. in diameter, presents a typical opaque central " spot," and, because of acid produced in the presence of bromcresol purple, is usually surrounded by a yellow halo in a field of purple. This halo may be insignificant, or missing, where certain low acid-producing types are concerned, or where the plate is so thickly seeded that the entire plate takes on a yellow tinge. The typical subsurface colonies are rather compact and may approach the " pinpoint " condition.

Where there is doubt as to the identity of the subsurface colonies, a decision can usually be made by observing the nature of the surface colonies. Where the surface colonies evidence reasonable purity of flora, it is safe for practical purposes to assume that the subsurface colonies have been formed by similar bacterial groups. It is emphasized that, where the plate is heavily seeded, there may be loss of accuracy as regards counts, and colony structure and size may be atypical. Where plates are so heavily seeded as to make counting impracticable, a second sample of the sugar may be plated, using dilutions of the original solution. For practical purposes, however, it is sufficient to note that the sample is obviously below standard.

[203] It is appreciated that the adequacy of this sampling will vary in relation to the size of the shipment or lot but it is felt that, where there is any significant variability in the shipment, this fact will become evident in the majority of cases, through individual tests on five samples. If subsequent experience shows it to be necessary, the sampling arrangement will be modified to the extent of taking the size of the lot into consideration.

[204] This medium may be obtained in dehydrated condition from the Difco Laboratories, Detroit, Mich., or from the various supply houses that carry Difco products in stock. Bacto-Dextrose Tryptone Agar was developed by the Difco Laboratories in collaboration with this laboratory with this special purpose in mind. It appears peculiarly adapted to growth of flat sour bacteria, and, in the medium, the colonies, both surface and subsurface, exhibit distinctive growth. Subsurface colonies are larger than in media formerly suggested. Further advantages lie in its standardization and convenience in preparation.

At times, the nature of subsurface colonies is in question. Whether they are flat sour colonies may often be determined by transferring, by the stroke method, from the colonies to agar plates. Their surface characteristics may then be noted. No spoilage significance is attached to the presence of " non-spoilage " thermophiles, i.e., aerobic spore-formers, actinomyces, etc., but when present in high number they carry the inference that the sugar has been produced or marketed under unsuitable conditions.

Detection of Thermophilic Anaerobes Not Producing H_2S. Divide 20 ml. of the sugar solution approximately equally among six liver broth tubes [205] and stratify the liquid medium with plain nutrient or yeast water agar. After the agar has solidified, preheat to 55° C. and incubate at that temperature for 72 hours.

Under the conditions stated, thermophilic anaerobes are manifest through the splitting of agar and the presence of acid. At times a " cheesy " odor is noted. The method is considered suitable as a qualitative test but quantitatively it provides only a means for estimation. The method does not permit expression of results in terms of numbers of spores per unit weight of sugar.

Detection of Thermophilic Anaerobes Producing H_2S (Sulfide Spoilage). This group is made up of the so-called sulfide spoilage organisms. Divide 20 ml. of the sugar solution approximately equally among six tubes containing " sulfite " agar.[206] Make inoculations in freshly exhausted deep agar tubes. Incubate at 55° C. for 72 hours.

In " sulfite " agar the " sulfide " spoilage organisms are detected through the formation of characteristic blackened spherical areas. Owing to the solubility of hydrogen sulfide and its fixation by the iron, no gas is noted. Certain of the thermophilic anaerobes (not producing H_2S), methods for the detection of which precede, give rise to relatively large amounts of hydrogen which splits the agar and reduces the sulfite, thereby causing general blackening of the medium. This condition, however, is readily distinguishable from the restricted blackened areas mentioned previously. The blackened areas may be counted to obtain quantitative results.

Reporting Results. Report total counts and flat sour and sulfide spoilage results as number of spores per 10 g. of sugar. Report thermophilic anaerobes (not producing H_2S) as number of tubes positive and number negative in the following manner: + + +, − − −.

[205] Five hundred grams of chopped beef liver is mixed with 1000 ml. of water and boiled slowly for 1 hour, after which the boiled material is pressed through cheesecloth and the liquid is made to 1000 ml. To the broth are added 10 g. peptone and 1 g. K_2HPO_4. The reaction is adjusted to *p*H 7.0. In tubing, ½ inch to 1 inch of the previously boiled ground beef liver is introduced into the tube.

[206] The formula for sulfite agar is: water, 1 liter; tryptone, 10 g.; sodium sulfite, 1 g.; agar, 20 g. At the time of tubing, a clean iron strip or nail is placed in the tube. No adjustment in reaction is necessary.

The following standards have been adopted:

Total Thermophilic Spore Count. For the five samples examined, there shall be a maximum of not more than 150 spores and an average of not more than 125 spores per 10 g. of sugar.

Flat Sour Spores. For the five samples examined, there shall be a maximum of not more than 75 spores and an average of not more than 50 spores per 10 g. of sugar.

Thermophilic Anaerobic Spores. These shall be present in not more than three (60 per cent) of the five samples and in any one sample to the extent of not more than four (65+ per cent) tubes.

Sulfide Spoilage Spores. These shall be present in not more than two (40 per cent) of the five samples and in any one sample to the extent of not more than five spores per 10 g. This would be equivalent to two colonies in the six inoculated tubes.

Refined sugars may be contaminated not only with thermophilic bacteria but also with ordinary mesophilic bacteria and with yeasts and molds. These organisms are not only detrimental to the keeping quality of the sugars, but may lead to serious trouble in soft drink and other food plants, as has been pointed out by Owen and Mobley.[207] These authors have proposed standard maxima for infection by organisms other than thermophilic bacteria, but this question requires further study.

STARCH PRODUCTS

Under this heading the following subjects will be taken up:

I. The determination of starch by methods not previously described.
II. The proximate composition of starch-conversion products.
III. The analysis of commercial dextrin gums.
IV. The analysis of malt extracts.
V. The determination of diastatic power.

I. Miscellaneous Methods for Determining Starch

A. POLARISCOPIC METHODS

The determination of starch with the aid of the polariscope was proposed by Dubrunfaut, and later again by Effront. Lintner developed a practical procedure in 1907,[208] and since then several methods have been devised for estimating starch from the polarization after conversion into soluble starch or into maltose.

[207] *Facts About Sugar,* **30, 451 (1935).**
[208] *Z. Untersuch. Nahr. u. Genussm.,* **14, 205 (1907).**

Method of Lintner. Two and a half grams of the finely ground material is rubbed in a mortar with 10 ml. of water and 15 to 20 ml. of concentrated hydrochloric acid. After standing for ½ hour, the mixture is washed into a 100-ml. flask with hydrochloric acid of 1.125 sp. gr. The albumen is precipitated by adding 4 per cent phosphotungstic acid, avoiding an excess (5 to 10 ml. is usually sufficient). The volume is completed with hydrochloric acid, sp. gr. 1.125, the solution filtered, and the filtrate polarized immediately. The soluble starch formed in this procedure has an average specific rotation of +202. Using this value, the weight of starch (*c*) in the 100 ml. solution is calculated from the angular rotation *a*, measured in a 200-mm. tube, by means of the formula

$$[\alpha]_D = \frac{100\,a}{c \times l}, \text{ whence grams starch } c = \frac{100\,a}{2 \times 202}.$$

A similar method has been published by Herles.[209]

Investigations by Schulz and Steinhoff[210] have shown that there is incipient hydrolysis of the starch if the temperature rises too high, or if the mixture of the product with the acid is allowed to stand too long. To avoid hydrolysis, the material, about 2 g., is rubbed with 10 ml. of water, and the mixture cooled to 4° C. Then 20 ml. hydrochloric acid, sp. gr. 1.19, is added, and the mixture is again cooled to 4° C. After 10 minutes' standing it is washed into a 100-ml. volumetric flask with hydrochloric acid, sp. gr. 1.125, the solution clarified with not more than 5 ml. of 4 per cent sodium phosphotungstate solution, the volume completed with hydrochloric acid, sp. gr. 1.125, the solution filtered and polarized. The calculation is made as in the original Lintner method, on the basis of +202 for the specific rotation.

Because of the irritating fumes of strong hydrochloric acid, Wenglein[211] substituted sulfuric acid of sp. gr. 1.7 and 1.3 for the hydrochloric acid, sp. gr. 1.19 and 1.125, respectively. Lintner[212] found, however, that this method is more subject to error from temperature rise than his method with hydrochloric acid. Later Schwarcz[213] reported satisfactory results in the analysis of barley, by treatment with sulfuric acid of sp. gr. 1.40 for 1 hour at 20° C., but according to

[209] *Orig. Com. 8th Intern. Congr. Appl. Chem.* (Appendix), 26, 5 (1913); *Z. Zuckerind. čechoslovak. Rep.*, 57, 256 (1932/33); 60, 298 (1935/36).

[210] *Z. Spiritusind.*, 55, 83 (1932).

[211] *Z. ges. Brauw.*, 31, 53 (1908).

[212] *Z. Untersuch. Nahr. u. Genussm.*, 16, 509 (1908); *Oesterr. Chem. Ztg.*, 15, 161 (1912).

[213] *Z. ges. Brauw.*, 36, 85 (1913).

Hopkins[214] different analysts may obtain widely divergent starch values by this method.

Method of Ewers.[215] In this method the use of strong acids is avoided entirely, but a higher temperature is employed. Five grams of the material, which must pass a 0.2-mm. screen, is intimately mixed in a mortar with 25 ml. of 1.124 per cent hydrochloric acid, and the mixture is washed into a 100-ml. volumetric flask with another 25-ml. portion of the same acid. The flask is placed in a boiling-water bath for 15 minutes, being agitated several times during the first 3 minutes. Enough cold water is added to complete the volume to about 90 ml., and the flask is cooled to 20° C. The solution is clarified with a minimum of phosphotungstic acid or sodium molybdate solution, made to the mark with water, filtered, and polarized. For the determination of starch in potatoes, 10 g. of air-dry material is used, and the strength of the hydrochloric acid is reduced to 0.4 per cent. Under the conditions of this method cereal starch has a specific rotation of +183, potato starch +195.4.

The method of Ewers has been recommended by von Scheele and Svensson[216] for rapid routine work, where only comparative results are desired. The specific rotation of various starches was found to vary from 180.9 to 183.5, and to average 182. If 4.745 g. of material is used, and the rotation of the solution is measured in a saccharimeter in a 200-mm. tube, the reading in degrees Ventzke, multiplied by 2, gives directly the percentage of starch, provided that the following corrections are applied: Deduct 1.1 for wheat or barley; 2.0 for maize; 3.8 for wheat bran; 0.5 for rye bran; 1.9 for barley bran; 3.0 for rice meal; add 1.2 for oats; 0.1 for rye; 2.4 for oat bran; 2.4 for oat meal.

Method of Mannich and Lenz, as Modified by Hopkins. Mannich and Lenz[217] have used a hot, concentrated calcium chloride solution to dissolve the starch, as first proposed by von Fellenberg.[218] To remove the soluble proteins, the extract is clarified with stannous chloride; or they are dissolved from another sample by extraction with cold calcium chloride solution, and the rotation found is deducted from that found for the extract prepared hot. Hopkins[219] found that neither of these procedures gives exact results, and that it is better first to remove optically active impurities by means of 65 per cent alcohol. The modi-

[214] *J. Assoc. Official Agr. Chem.*, **22**, 523 (1939).
[215] *Z. öffentl. Chem.*, **15**, 8 (1909).
[216] *Tek. Tid., Uppl. C, Kemi*, **58**, 57, 65 (1928).
[217] *Z. Untersuch. Nahr. u. Genussm.*, **40**, 1 (1920).
[218] *Mitt. Lebensm. Hyg.*, **7**, 369 (1916).
[219] *Can. J. Research*, **11**, 751 (1934).

fied method is carried out as follows. Two to 2½ g. of the sample, which must be ground fine enough to pass a 100-mesh sieve, is weighed into a round-bottom, 50-ml. centrifuge tube, with lip. Ten milliliters of alcohol of specific gravity 0.88 at 20° C. is added, and the mixture is thoroughly stirred with a glass rod. The rod is placed aside carefully to be used again, the tube is centrifuged, and the supernatant liquid is poured off. The extraction treatment is repeated with five further 10-ml. portions of alcohol, the same glass rod being used as before. The final residue is stirred with 10 ml. of water, the mixture poured into an Erlenmeyer flask of about 125-ml. capacity, and transferred quantitatively by means of 60 ml. of concentrated calcium chloride solution (sp. gr. 1.3, from 2 parts $CaCl_2 \cdot 6H_2O$ and 1 part of water made alkaline to phenolphthalein with NaOH) containing 2 ml. of 0.8 per cent acetic acid. The glass rod is also placed in the flask, and the mixture is brought quickly to boiling over a wire gauze, with frequent stirring. Boiling is continued for 15 to 17 minutes, the flame being regulated so as to prevent foaming or burning. Particles adhering to the wall of the flask are rubbed down from time to time with the glass rod. The flask is then quickly cooled in running water, the contents are poured into a 100-ml. volumetric flask and washed into it with concentrated calcium chloride solution, finally making up to the mark with it. Foam may be destroyed with a drop of alcohol. The flask is well shaken, about 10 ml. of the contents is poured on a fluted filter (Whatman No. 42 or 44), completely wetting the paper, and when it has passed through the filter, this part of the filtrate is discarded. The remainder of the mixture is filtered into a dry flask until about 40 or 50 ml. has accumulated. The angular rotation of this filtrate is measured in a 100-mm. tube, two sets of ten readings each being taken. The results of the two sets should check within 0.006°. The per cent starch is then calculated by the formula

$$\frac{100 \times 100\,a}{200\,s} = \frac{50\,a}{s}$$

where a is the reading in a 100-mm. tube, and s the weight of material taken. This formula is applicable only to wheat starch and wheat products.

Method of von Scheele and Svensson.[220] In this method the starch is treated with diastase under specified conditions, by a procedure first used by Ling, Nanji, and Harper,[221] and modified by Lüers and Wien-

[220] *Tek. Tid., Uppl. C, Kemi,* **58**, 57, 65 (1928).
[221] *J. Inst. Brewing,* **30**, 838 (1924).

inger.[222] The former authors determined the copper-reducing power of the conversion products; the latter employed oxidation with hypoiodite for the purpose. Polarimetric estimation, as advocated by von Scheele and Svensson, is much more rapid, however.

Three grams of the finely ground material is gently boiled under reflux for 30 minutes with 100 ml. of water in a 200-ml. volumetric flask, and 100 ml. of Sørensen's phosphate buffer mixture of 6.24 *p*H is added. This buffer solution is prepared by mixing 2 ml. of a solution containing 11.876 g. $Na_2HPO_4 \cdot 2H_2O$ per liter, with 8 ml. of a solution containing 9.078 g. KH_2PO_4 per liter. After the mixture has been cooled to 65° C., 0.1 g. of Diastase Absolute (Merck), which has been rubbed up with 5 ml. of water, is added and transferred quantitatively by washing twice with 2.5 ml. of water each. The flask is incubated at 63° C. for 2 hours, with occasional shaking. The mixture is boiled once more under reflux for 30 minutes, cooled to 65° C., another 0.1 g. of diastase is added as before, the flask incubated again at 63° C. for 30 minutes, and the contents brought to boiling and cooled to room temperature. The solution is acidified with 10 ml. of *N* hydrochloric acid, clarified with 3 to 10 ml. of 4 per cent phosphotungstic acid solution, made to volume, shaken, and filtered. The polarization is measured in degrees Ventzke in a 200-ml. tube.

To correct for the optically active impurities present, a blank determination is run by covering 15 g. of the original material in a beaker with absolute alcohol to the depth of 1 cm., and placing the beaker, covered with a watch glass, on a water bath for 30 minutes, constantly replacing the evaporated alcohol. The alcohol is then boiled off, and the residue dried for 30 minutes at 100° C. It is then transferred to a 400-ml. beaker with 250 ml. of water, the mixture is allowed to stand for 1 hour with frequent stirring, and filtered. Fifty milliliters of the filtrate, corresponding to 3 g. of the original material, is diluted to 100 ml. in a 200-ml. volumetric flask. This solution is treated exactly like the original extract obtained by boiling 3 g. of the substance under reflux, the final filtrate is polarized in a 200-mm. tube, and the reading is deducted from the reading obtained before. The corrected polarization of 3 g. of pure, dry wheat starch, treated as described, is +13.8° V., and the percentage of starch in the unknown is calculated by simple proportion. Pentosans were found not to affect the determination.

The polariscopic methods described, although well recommended by some authors, have not found general acceptance, and the complete hydrolysis methods, described on pp. 858–865, are considered to be more reliable, especially for complex starch products.

[222] *Z. ges. Brauw.*, June, 1925.

B. GRAVIMETRIC METHODS

Method of Rask for Determination of Starch in Flour. Rask[223] discovered that starch is dispersed by cold, moderately strong hydrochloric acid to a filterable solution, without noticeable chemical change, and can be reprecipitated by alcohol. A modification of Rask's method has been adopted tentatively by the Association of Official Agricultural Chemists for the determination of starch in wheat flour.[224] It requires great attention to every detail, and is therefore described in full:

Mix approximately equal volumes of strong hydrochloric acid and water, and adjust by titration so that 100 ml. of the solution contains 20.5 to 21.0 g. of HCl.

Weigh accurately a sufficient quantity of finely ground sample (should readily pass through a 20-mesh sieve) to represent 0.5 to 1.0 g. of starch. The quantity of starch finally weighed will then vary from 0.25 to 0.5 g. Transfer to a funnel fitted with a filter paper (9 cm. S. and S. No. 589 White Ribbon, or Whatman No. 40), and extract by nearly filling the filter four times with ethyl ether; likewise extract with 70 per cent (by volume) alcohol, and with water. Allow to drain 1 hour uncovered. Transfer the drained filter and contents to a 50-ml. beaker. The stirring rod to be used in the next step should have a flattened end 15 mm. in diameter. (It is very important to tamp with a twisting motion during the time specified below in order to get the filter paper completely disintegrated and thus insure the complete suspension of the starch in the hydrochloric acid solution. This time should be sufficiently long and the maceration complete to allow the suspension of all the starch but not to hydrolyze any of it. Maceration should be completed while there is a small amount of hydrochloric acid present and the whole contents are a rather thick paste. If this optimum condition is obtained practically duplicate results will follow. Add the hydrochloric acid reagent at 15° C. to the beaker containing the sample, using a fast-delivering 10-ml. Mohr pipette with 1-ml. divisions marked off at the lower end with heavy pencil marks. Keep the acid supply on the bench, but do not allow it to get above 18° C.)

Proceed as follows, adding the hydrochloric acid in the quantities given: Add 1 ml., tamp 1 minute; add 1 ml., tamp 2 minutes; add 1 ml., tamp 2 minutes; add 1 ml., tamp 1 minute; add 1 ml., tamp 1 minute; add 1 ml., tamp 1 minute. Fill beaker half full with the acid and stir 30 seconds; fill beaker three-fourths full and stir 30 seconds.

In ten minutes during this treatment the paper should be completely disintegrated and in a smooth state of suspension, the tamping should be continued vigorously during this time, and as little time as possible should be spent adding the acid. Transfer immediately to a 100-ml. wide-mouthed

[223] *J. Assoc. Official Agr. Chem.*, **10**, 108 (1927).

[224] *J. Assoc. Official Agr. Chem.*, **16**, 504 (1933); "Methods of Analysis, A.O.A.C." 5th ed., pp. 221–222, 1940.

(Kohlrausch) volumetric flask, rinsing out the beaker with the acid, carefully make to volume with hydrochloric acid reagent; and add 0.5 ml. for volume of filter paper (this step requires 2 minutes). Next shake the stoppered flask vigorously for 5 minutes, then allow to stand 5 minutes in a beaker of water at 20° C. Shake twice and filter immediately into a 250-ml. suction flask through a small Büchner funnel (41 mm. in diameter) fitted with a thin layer of asbestos and filled half full with dry, fluffy asbestos. The filtration requires 1 minute only. Pipette immediately 50 ml. of the filtrate into a 200-ml. beaker (tall form) containing 115 ml. of 95 per cent alcohol (by volume). (The time consumed from the initial addition of the acid to the sample is 24 minutes.) Allow the pipette to drain completely, and then stir with a whipping motion for 1 minute to flocculate the precipitated starch. Wash down the sides of the beaker with 70 per cent alcohol. Allow to stand 3 or 4 minutes, until nearly all the precipitate has settled, and then carefully decant the supernatant liquid, which is somewhat turbid, so that little or no precipitate passes into the weighed Gooch crucible, which has been fitted with a thin pad of ignited asbestos and is half filled with fluffy ignited asbestos. Wash the precipitate and filter by decantation, using successively two 40-ml. portions of 70 per cent alcohol (by volume), then four times, using about 30-ml. portions of 95 per cent alcohol (by volume), each time breaking up the precipitate by rapid stirring and allowing the precipitate to settle before decantation. After each stirring rinse the sides of the beaker with a small stream of alcohol to prevent the starch from drying and sticking to the sides of the beaker. Finally transfer the starch completely by means of a jet of 95 per cent alcohol (by volume) and wash the sides of the Gooch and precipitate with a little of the alcohol. (All these filtrations are very fast.) Dry the crucible and contents uncovered for 2 hours at 130° C.; cover the crucible immediately and place in a desiccator charged with phosphorus pentoxide, or fresh concentrated sulfuric acid, or freshly ignited CaO; cool 10 minutes, and weigh. Multiply result by 2 and report as starch. These directions must be followed carefully in every detail to obtain satisfactory results. Since the steps are timed it is essential to learn the procedure, so that no time will be lost in following it through. Arrange everything needed in the determination before the hydrochloric acid is added to the sample.

Denny[225] tried the Rask method on a number of plant materials and found that the starch is in some cases embedded so firmly in the cells that one extraction with acid is not sufficient. On the other hand, the alcohol precipitate often contained non-starch substances, precluding direct gravimetric determination. It is therefore necessary to make several extractions with acid, and to determine the starch in the precipitate, preferably by the takadiastase method (p. 861). Sulfuric acid may be used for the extractions, instead of hydrochloric acid. The extraction is facilitated by first gelatinizing the starch.

[225] *Contrib. Boyce Thompson Inst.*, 6, 381 (1934).

Separation of Starch by Means of Iodine. Precipitation of the starch as iodine-starch complex from a calcium chloride extract of the material was introduced as a quantitative method by von Fellenberg.[226] The iodine was removed with alcohol, and the remaining starch weighed directly. Denny[227] made a study of this method also, and at first determined the iodine in the complex by means of thiosulfate. In a later modification[228] the starch is extracted by four treatments with concentrated calcium chloride solution, the starch precipitated with iodine-potassium iodide solution, the iodine volatilized by boiling with water, and the starch hydrolyzed with takadiastase. The results by this method closely approximate those of the Rask method as modified by Denny, but the values obtained by either of them are usually lower than those found by the takadiastase or the Walton and Coe method (p. 859), indicating that in the latter methods non-starch constituents are determined as starch. Other procedures in which the starch is first extracted with either calcium chloride solution or with hydrochloric acid have been published by Sullivan[229] and by Pucher and Vickery.[230] Both these methods are very time consuming. Moreover, Chinoy[231] contends that they give erroneous results because of partial hydrolysis of the starch; he prefers extraction with 0.7 per cent potassium hydroxide solution. After neutralization the starch is precipitated with iodine in the presence of potassium acetate, the precipitate is treated with alcohol, and the starch is weighed as such.

Reliability of Methods for Determining Starch. The very fact that so many different methods have been proposed for this determination indicates that a procedure applicable in all cases is yet to be devised. A method may give satisfactory results with a certain class of material, but fail in other instances. According to Hopkins,[232] the methods of Denny, of Sullivan, and of Pucher and Vickery, developed for the analysis of plant materials low in starch, do not appear to be applicable to products of medium or high starch content. The Mannich-Lenz method, on the other hand, is unsuited for cottonseed meal, low in starch, but gives good results for commercial wheat starch. The problem is aggravated by the fact that in many cases there is no absolute standard by which the accuracy of a method may be judged.

[226] *Mitt. Lebensm. Hyg.*, 7, 369 (1916); see also p. 863.
[227] *J. Assoc. Official Agr. Chem.*, 6, 175 (1922/23).
[228] *Contrib. Boyce Thompson Inst.*, 6, 381 (1934).
[229] *J. Assoc. Official Agr. Chem.*, 18, 621 (1935).
[230] *Ind. Eng. Chem., Anal. Ed.*, 8, 92 (1936).
[231] *Analyst*, 63, 876 (1938).
[232] *J. Assoc. Official Agr. Chem.*, 22, 523 (1939); see also Sullivan, *Ind. Eng. Chem., Anal. Ed.*, 7, 311 (1935).

II. Proximate Composition of Starch-Conversion Products

Brown, Morris, and Millar[233] have shown that in starch products of diastase conversion a constant relation exists between the specific rotation and copper-reducing power of the total solids. Rolfe and Defren[234] have also shown that in starch products of acid conversion the solids of same specific rotation have always the same reducing power "irrespective of the source of the starch, the nature or amount of the hydrolyzing acid, or the temperature conditions, these influencing the *rate* of hydrolysis only." It is possible, therefore, to express by means of

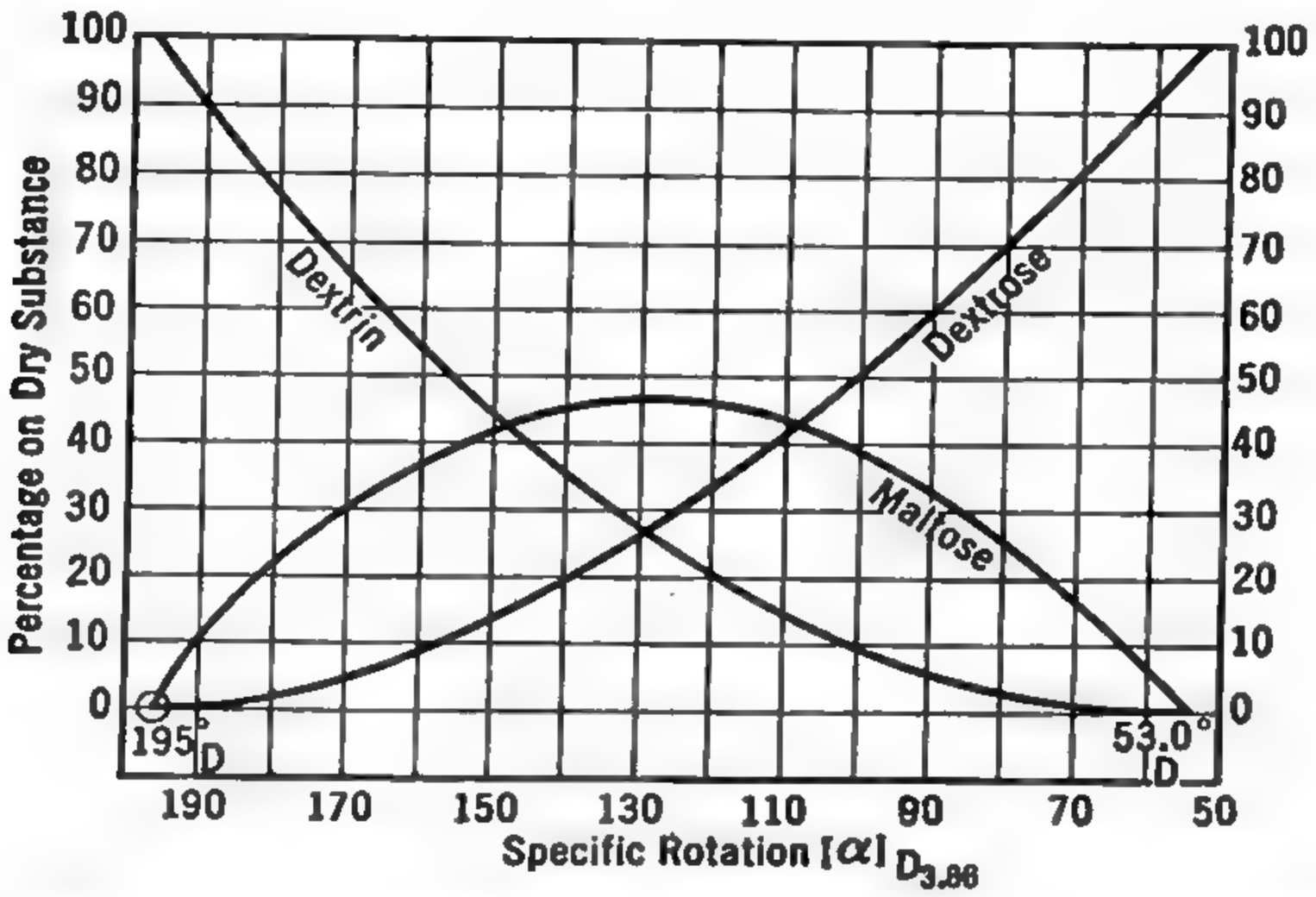


Fig. 329. Showing relation of specific rotation to composition of acid-hydrolyzed starch products.

a curve the relationship between specific rotation and copper-reducing power, or between either of these constants and the apparent percentages of glucose, maltose, and dextrin, calculated by means of such formulas as are used in Allen's method (p. 989). Upon this principle Rolfe has prepared the diagram shown in Fig. 329, which gives the percentages of dextrose, maltose, and dextrin in the dry substance of starch-conversion products corresponding to the values of $[\alpha]_D$ for dry substance (as determined by the solution factor 3.86) between +195 for dextrin and +53 for glucose.

A value, for example, of $[\alpha]_D = +100$ for the dry substance (calculated from the density of an approximately 10 per cent solution at 15.5° C. by the solution factor 3.86) of an acid-conversion product would correspond to an apparent composition of dry substance of 10 per cent dextrin, 40 per cent maltose, and 50 per cent glucose.

[233] *J. Chem. Soc.*, 71, 115 (1897).

[234] *J. Am. Chem. Soc.*, 18, 869 (1896); Rolfe, "The Polariscope," p. 197, 1905.

The apparent percentages as thus determined are useful for purposes of comparison and valuation but must not be mistaken for absolute percentages for reasons already given. As Rolfe is careful to state, "There are comparatively few commercial products pure enough to permit of their constitution being determined in this simple manner."

III. Analysis of Commercial Dextrin Gums

Method of Browne and Bryan. The following method has been used by the United States Bureau of Chemistry in testing dextrins for the National Bureau of Printing and Engraving. The method is a modification by Browne and Bryan[235] of a scheme of analysis proposed by F. Lippmann.[236]

Specific Rotation. Transfer 10 g. of the finely divided sample to a 100-ml. flask, and after solution in about 50 ml. of cold water add 5 ml. of alumina cream and make up the contents to 100 ml., thoroughly shake, and filter. Polarize the filtrate in a 200-mm. tube, using any form of polariscope or saccharimeter. It is important that a 6 per cent solution of bichromate of potash in a 3-cm. tube be used as a light filter. In using a Ventzke-scale saccharimeter, the specific rotation is found by the formula $[\alpha]_D = \frac{34.68 \times V}{20}$, in which V = Ventzke reading.

Viscosity. Dissolve 100 g. of dextrin in 200 ml. of cold water by rubbing up in a mortar or porcelain dish, and determine the viscosity of the solution by any of the standard forms of viscosimeter. Comparative tests should always be made with the same instrument and under similar conditions of temperature; a uniform length of time should also elapse after making up the solution before taking the viscosity. The viscosity should be determined again on the same solution after standing 24 hours, and also after 48 hours.

Moisture. Determine by drying from 2 to 5 g. of sample for 4 hours at a temperature of 105° C. Absolute constancy in weight cannot be attained on account of the slow decomposition of the dextrin.

Ash. Five to 10 g. of the sample is weighed in a tared platinum dish and burned over a flame at a low heat. The ash should not be heated to fusion, otherwise loss from volatilization will occur.

Soluble Starch. If a filtered hot-water solution of the dextrin gives a blue reaction with iodine solution, soluble starch is indicated. Weigh two lots of dextrin, 10 g. each, into 100-ml. flasks, add 50 ml. of cold

[235] *Proc., Sec. V, Seventh Int. Cong. App. Chem., London,* p. 337, 1909.
[236] *Z. Spiritusind.*, 25, 304, 307, 316, 317 (1902).

water to each, and after all soluble matter is dissolved make up the contents of the one flask with cold water at 100 ml., shake, and filter. Evaporate 20 ml. of the solution (2 g.) to dryness and dry for 4 hours at 105°, as under determination of moisture. Weight of residue, less ash on incineration, equals cold-water-soluble organic matter. Heat the contents of the second flask to boiling, and then after cooling make up to 100 ml., shake, and filter. The weight of hot-water-soluble organic matter in 20 ml. of solution is determined as before. Hot-water-soluble organic less cold-water-soluble organic gives the soluble starch.

Unconverted Starch. If the residue insoluble in hot water shows under the microscope grains which are colored blue with iodine, unconverted starch is present. To determine the percentage, collect the residue insoluble in hot water on a filter, wash until free from soluble matter, and determine the starch by the usual methods.

Reducing Sugars. Determine in an aliquot of the cold-water soluble by the method of Allihn, the results being expressed as glucose.

Dextrin. Subtract the specific rotation of the dextrin due to reducing sugars $\frac{(52.5 \times \text{per cent reducing sugar as glucose})}{100}$ from the original specific rotation of the sample. Multiply the remainder by 100 and divide by 186 ($[\alpha]_D$ of dextrin[237]) to obtain the calculated percentage of dextrin in the sample.

Undetermined Solubles. The percentage of cold-water-soluble organic matter less calculated percentage of dextrin gives the percentage of undetermined solubles.

In Table CXXXVII eight analyses of commercial dextrins by the above method are given.

It is noted that, with a decrease in specific rotation, there is a uniform decrease in viscosity and in the calculated percentage of dextrin and a uniform increase in reducing sugars and undetermined matter. A large percentage of reducing sugars indicates over-dextrinization, and accompanying this there is always a formation of other decomposition products.

The viscosity determination is of paramount value as a physical test in examining the qualities of dextrins, likewise the change in viscosity of the cold-water solution after 24 hours' and 48 hours' standing. In the technical application of dextrins such an increase in viscosity, if

[237] The $[\alpha]_D$ +186 of dextrin is given by Schulze (*J. prakt. Chem.*, **28**, 327). This is considerably lower than the figures +195 to +205, which have been reported by other authorities for carefully purified dextrins. The value +186 is used only as a commercial standard of comparison, and the percentages of dextrin thus calculated have no strict scientific value.

TABLE CXXXVII

Analyses of Commercial Dextrins

No.	$[\alpha]_D$	Viscosity at 20° C. 1 to 2 Solution Water = 100				Chemical Analysis							
		Cold-Water Solution		Hot-Water Solution		Moisture at 105°C.	Ash	Reducing Sugars as Glucose	Cold-Water-Insoluble Organic Matter	Dextrin		Undetermined Soluble Matter	Acidity 0.1 *N* KOH per 10 g.
		Immediate	After 24 hours	Immediate	After 24 hours					By Difference	From Polarization		
						per cent	per cent	per cent	per cent	per cent	per cent	per cent	ml.
1	+175.2	844	1332	396	428	2.92	0.09	1.80	0.24	94.95	93.74	1.21	2.2
2	+174.1	620	980	396	430	3.96	0.08	1.77	0.34	93.85	93.15	0.70	2.0
3	+172.7	596	860	480	480	2.88	0.14	1.56	0.45	94.97	92.46	2.51	2.6
4	+167.5	480	692	242	256	4.46	0.16	2.44	1.95	90.99	89.43	1.56	2.3
5	+163.7	420	636	324	330	6.07	0.09	2.20	0.31	91.33	87.45	3.88	2.5
6	+162.2	384	448	348	370	4.76	0.13	2.03	3.37	89.71	86.69	3.02	2.0
7	+159.2	344	392	240	260	2.39	0.14	5.59	3.27	88.61	84.16	4.45	4.0
8	+149.8	300	332	184	186	4.42	0.13	5.78	2.48	87.19	79.07	8.12	5.3

large, will overtax the machines or impair the results of the work. The figures in the table corroborate the views of Lippmann that the cold-water solution only should be used for the viscosity test, since the individual differences between dextrins are thus rendered more distinguishable than where the solutions are made in hot water.

Method of Babington, Tingle, and Watson.[238] In this method the starch in commercial dextrins is precipitated by a strong barium hydroxide solution, and the dextrin-gum is determined in the filtrate. One gram of the material is gelatinized by warming with 30 ml. of water in a 100-ml. flask, 50 ml. of a saturated solution of barium hydroxide is added with constant agitation, the volume is completed, and the solution filtered. Fifty milliliters of the filtrate is pipetted into a platinum dish, acidified slightly with *N*/10 hydrochloric acid and phenolphthalein as indicator, and made faintly alkaline again by the addition of 1 or 2 drops of barium hydroxide solution. Ten grams of dry, ignited sand is added, and the dish is heated on the water bath with constant stirring until the contents are almost dry. Drying is continued in an oven at 120° C., until constant weight is obtained. The residue is ashed at a low temperature with stirring, and reweighed. The difference between the dry substance and the ash represents the dextrin-gum in 0.5 g. of material. The accuracy of the method is about 5 per cent.

Trotman[239] has used the above method in the analysis of commercial dextrins. The ash, moisture, insoluble starch, and reducing sugars are

[238] *J. Soc. Chem. Ind.*, 37, 257T (1918).
[239] *Dyer and Calico Printer*, 60, 36, 59 (1928).

determined by the usual methods. Then the per cent of total starch is the difference between 100 and the sum of ash, moisture, and dextrin-gum. The soluble starch is total starch less insoluble starch. The non-reducing dextrin-gum is dextrin-gum minus reducing sugars, expressed as glucose. Dextrin is estimated by hydrolyzing the cold-water extract with acid, determining the reducing sugars, and deducting the reducing sugars originally present, both expressed as glucose; the result is multiplied by 0.9. The dextrin may also be determined by the method described on p. 1134.

Caesar and Cushing[240] have shown that the method of Babington, Tingle, and Watson, though useful for purposes of classification, is purely arbitrary; if more barium hydroxide than the quantity prescribed is used the percentage of " starch " increases, and that of " dextrin-gum " decreases. The resistance of starch against degradation by potassium hydroxide, measured by the procedure of Taylor and Salzmann,[241] is a much better criterion for differentiating between starch and dextrin.

Method of Edwards, Nanji, and Chanmugam.[242] These authors also found the method of Babington, Tingle, and Watson to be unsatisfactory, and obtained better results by precipitating the starch as the starch-iodine complex. To determine the dextrin, not more than 1 g. of the commercial dextrin is rubbed to a paste with 5 ml. of water in a beaker. It is dissolved by slowly adding 100 ml. of hot water with stirring, and simmering gently for ½ hour. The hot solution is washed into a 200-ml. volumetric flask, cooled, and made to the mark. A 20-ml. aliquot of this solution is pipetted into a 100-ml. flask, 2 ml. of 0.1 *N* iodine solution is added, and the volume is completed with a solution containing 100 ml. of 50 per cent alcohol (by volume) and 4 ml. of 10 per cent potassium acetate solution. The mixture is well shaken, allowed to stand for 5 minutes, and filtered. Fifty milliliters of the filtrate (= 10 ml. of the original solution) is evaporated to about 3 to 4 ml. After cooling, the dextrin is precipitated by the addition of 100 ml. of 95 per cent alcohol with constant stirring. After standing overnight, the dextrin is filtered off through an alundum crucible of medium porosity, washed with 95 per cent alcohol, dried, and weighed.

For the starch determination, not more than 1 g. of the sample is gelatinized with hot 0.7 per cent potassium hydroxide solution, dissolved in hot water, the solution cooled and diluted to 200 ml. Ten milliliters of this solution is neutralized with acetic acid, phenolphthalein being used as indicator, 1 ml. of 0.1 *N* iodine solution is added, and

[240] *Ind. Eng. Chem.*, **31**, 921 (1939).
[241] *J. Am. Chem. Soc.*, **55**, 264 (1933).
[242] *Analyst*, **63**, 697 (1938).

then 40 ml. of the alcohol and potassium acetate solution specified above. The mixture is allowed to stand for 10 minutes, the liquid is decanted from the precipitate in the beaker through an alundum crucible, the precipitate washed in the beaker twice with 50 per cent alcohol and then twice with 95 per cent alcohol, transferred to the crucible, washed with 95 per cent alcohol, dried, and weighed.

According to Grossfeld and Hollatz,[243] starch may be separated from dextrin by precipitation with zinc ferrocyanide, produced in the solution by adding equivalent quantities of potassium ferrocyanide and zinc acetate. The precipitate is filtered off, and the dextrin determined in the filtrate by precipitation with alcohol.

IV. Analysis of Malt Extracts

Malt extracts are employed by bakers for the improvement of bread, in the textile industry for the removal of starch from raw materials or fabrics, as a special food material for infants or dietary purposes, and also in the manufacture of certain beverages. The extracts are prepared by evaporating the filtered wort from mashed malt to a sirup. Malt extracts used in the baking and textile industries are valued for their diastatic power. The temperature at which the extracts are made has an important influence on their composition. If they are made in the cold, the percentage of maltose will be low, and that of the sugars preexisting in the malt as invert sugar and sucrose will be high. If the malt is mashed at 60° C., then the extract will contain a large excess of maltose due to the conversion of the starch by the diastase. The following analyses by Jago[244] show the marked difference in composition between extracts made by cold-water and warm-water mashing.

TABLE CXXXVIII

Constituent	Cold-Water Mash		Warm-Water Mash, 60° C.	
	Extract, Unevaporated	Extract, Evaporated	Extract, Unevaporated	Extract, Evaporated
Water	95.17	22.90	86.70	14.70
Ash	0.32	4.80	0.24	1.70
Proteids	0.80	12.71	0.86	5.27
Dextrin	0.60	13.66	1.32	10.82
Sucrose	0.45	4.79	0.43	0.00
Maltose	0.21	2.69	9.04	60.97
Glucose and fructose	2.45	38.45	1.41	6.54
	100.00	100 00	100.00	100.00

[243] *Z. Untersuch. Lebensm.*, **59**, 216 (1930).

[244] "The Technology of Bread Making," p. 512, 1911.

In the analysis of such a complicated mixture of sugars and other carbohydrates as occurs in malt extracts, malt sirups, and maltose sirups the chemist must employ indirect methods, the use of which involves a considerable multiplication of experimental errors as previously explained (p. 991). Several such methods are described in Chapter XVI. The results thus obtained have only an approximate value. The extracts must be carefully clarified in order to eiiminate the influence of soluble proteids.

Fermentation Method of Schultz and Kirby.[245] These authors have described a procedure for the determination of glucose or fructose, sucrose, and maltose in mixtures, which has proved particularly valuable for the analysis of malt extracts. The method, which has been adopted by the American Association of Cereal Chemists,[246] is carried out as follows.

The apparatus[247] is shown in Fig. 330. It consists of six 200-ml. wide-mouthed reaction bottles, *A*, immersed in a thermostat at 30° C. and attached by means of spring clamps to a cradle of strap iron. The cradle is continuously rocked through an arc of about 20° by a small motor at the rate of about 120 oscillations per minute. Each bottle is connected by rubber tubing with a 1-liter gas burette (*B*). The water replaced by the gas is collected in a liter flask, *C*, which also serves as a leveling device. A layer of kerosene reduces solution of carbon dioxide in the water to a negligible minimum.

The *p*H of the fermenting solutions is held approximately constant near the optimum point by means of a buffer solution containing 100 g. potassium citrate, 20 g. citric acid, and 20 g. primary ammonium phosphate per liter, and sterilized by boiling. The solution to be analyzed should contain not more than 4 g. fermentable sugar.

Determination of Glucose and Fructose. A pure culture of a mycoderma organism which ferments these two sugars, but not sucrose or maltose, is employed. In order to acclimatize the organism and to saturate the solution with carbon dioxide, 1 g. of pure glucose is weighed into the reaction bottle, and 25 ml. of buffer solution is added. Five grams of the mycoderma organism is weighed into a beaker, creamed with water, and washed into the reaction bottle with a total of 75 ml. of water. The reaction bottle is then placed in the cradle and connected with the gas burette. Shaking is commenced and continued until the original charge of glucose is completely fermented out, as shown by the constancy of successive readings on the burette over two 15-minute periods.

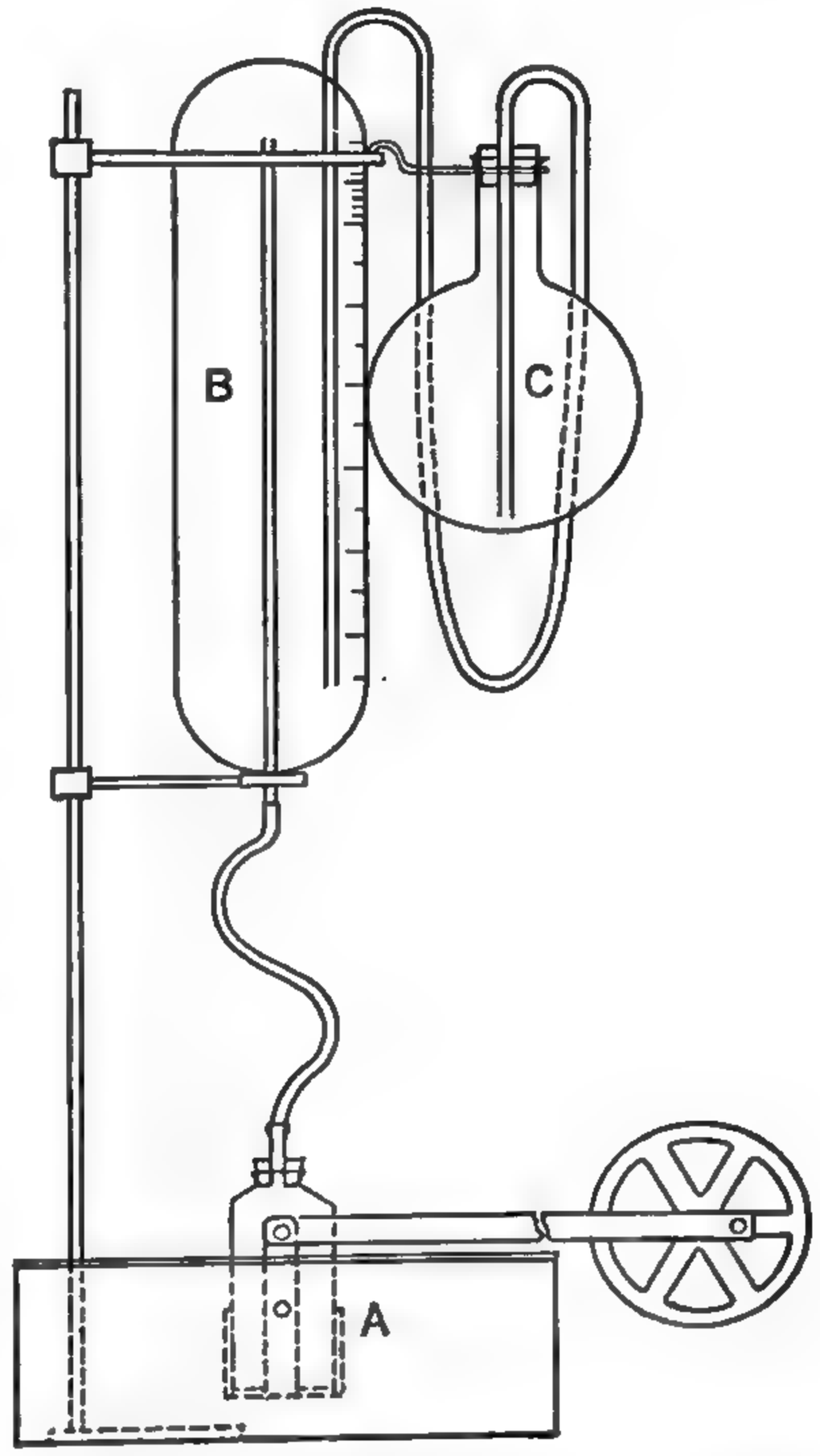
The shaking is then stopped, the burette reset to zero, a weighed sample of the malt extract is added, and the zero reading noted. Shaking is resumed

[245] *Cereal Chem.*, 10, 149 (1933).
[246] "Cereal Laboratory Methods," 3d ed., p. 61, 1935.
[247] *J. Am. Chem. Soc.*, 54, 212 (1932).

and continued until fermentation is complete as indicated by the constancy of the burette readings, observed as before. The volume of carbon dioxide obtained, divided by the volume of carbon dioxide evolved from 1 g. of pure standard glucose in a parallel experiment, equals the number of grams of glucose or fructose in the sample taken.

Determination of Sucrose. After the first fermentation is completed, shaking is stopped, the burette reset to zero, and 2 ml. of a 5 per cent solution of commercial invertase preparation added. The zero reading is noted, and the shaking is resumed until the sucrose is completely fermented as indicated by constant reading. The volume of carbon dioxide thus obtained, divided by the volume of that obtained from 1 g. of pure sucrose in a parallel experiment, equals the number of grams of sucrose present.

Total Fermentable Sugars. In this instance 3 g. of fresh baker's yeast is used, and acclimatization is carried out with 1 g. of pure maltose. When fermentation is complete, shaking is stopped, the burette reset to zero, and a weighed sample of the material to be analyzed, containing about 3 g. but not more than 4 g. of total sugar, is added to the reaction bottle, which is connected to the burette as quickly as possible, given a few whirls by hand, and the zero reading is taken. Shaking is resumed and continued until fermentation is complete, and the volume of carbon dioxide is noted. Parallel experiments are carried out at the same time to determine the volume of carbon dioxide obtained per unit weight of pure glucose, sucrose, and maltose with baker's yeast.



(Reproduced with permission from J. Am. Chem. Soc., 54, 212.)

FIG. 330. Apparatus for fermentation method of Schultz and Kirby.

The calculations are made as shown in the example below. Since the maltose is obtained by difference, the result is less reliable than that for sucrose and glucose plus fructose.

It is best to start the experiment early in the morning so that it can be completed the same day. A smaller sample of malt extract is used for the determination of the total sugars because the amount of carbon dioxide is much larger.

In a typical analysis of malt extract Schultz and Kirby obtained the following record.

	Time	1 Mycoderma	2 Baker's Yeast
		ml. gas	ml. gas
Acclimatization period, 1 g. glucose or maltose	9.45	0	0
	11.15	125	140
	11.30	140	155
	11.45	140	155
10 g. malt sirup added to No. 1, 5 g. malt sirup added to No. 2	11.45	150	160
	12.15	220	260
	12.45	305	...
	1.00	320	...
	1.15	320	610
Invertase added to No. 1	1.16	340	...
	1.45	370	...
	2.00	375	...
	2.15	375	660
	2.30	...	665
	2.45	...	670
	3.00	...	670

Hence 320 − 150 = 170 ml. carbon dioxide obtained from glucose in 10-g. sample.

375 − 340 = 35 ml. carbon dioxide obtained from sucrose in 10-g. sample.

670 − 160 = 510 ml. carbon dioxide obtained from fermentable sugars in 5-g. sample or 1020 ml. from 10-g. sample.

Under the same conditions pure glucose and sucrose each gave 200 ml. carbon dioxide per gram with the mycoderma organism. With baker's yeast glucose and maltose each gave 200 ml. per g., sucrose 210 ml.

Therefore the 170 ml. carbon dioxide obtained from the glucose in 10 g. malt sirup corresponds to 0.85 g. glucose, and the 35 ml. carbon dioxide from the sucrose to 0.175 g. sucrose. With baker's yeast the 0.85 g. glucose also gives 170 ml. carbon dioxide, but the 0.175 g. sucrose gives $0.175 \times 210 = 37$ ml. Deducting the sum 207 ml., from the 1020 ml. obtained from the total fermentable sugars gives 813 ml. obtained from maltose, equal to 4.065 g. maltose. The malt extract thus contained 8.5 per cent glucose, 1.75 per cent sucrose, and 40.65 per cent maltose.

The temperature of the thermostat should not fluctuate more than 0.5° C. If the atmospheric pressure should vary during the course of an experiment, the necessary corrections must be applied to the volume of the carbon dioxide. In the analysis of a dilute sugar solution it is

not necessary to acclimatize the organisms, but carbon dioxide must be passed through the solution in order to saturate it with this gas.

V. Determination of Diastatic Power

Diastase is a group name for starch-splitting enzymes of plant and animal origin, more specifically called amylases. The amylases in ungerminated and malted grains have been extensively studied, and it has been found that they may be characterized by three well-defined types of activity, saccharogenic, liquefying, and saccharifying.[248] The saccharogenic activity is manifested by attack on raw starch, with the formation of reducing sugars; the liquefying activity brings about a rapid fall in the viscosity of starch paste, through dextrinization; the saccharifying power is shown by conversion of soluble starch into maltose. It is not definitely known whether each of these actions is due to one or several enzymes, but the existence of at least two is quite generally accepted, although opinions differ as to their exact functions.

Ungerminated grain exhibits mainly saccharogenic power, and this is ascribed to the effect of β-amylase, which is supposed to split off a maltose molecule from the end of each polyglucoside chain. The reaction rate of saccharogenesis is very slow. Liquefying power is characteristic of sprouted grain. It is considered to be due principally to the effect of α-amylase which breaks glucose linkages in the interior of the chain and converts the starch into dextrins with progressively shorter chains. The reaction rate of liquefaction is much faster than that of saccharogenesis. Saccharification is brought about by both α- and β-amylase. It appears that in a medium dextrinized by α-amylase the β-amylase becomes more active because it has more points of attack presented to it. It is also probable that sprouted grain contains substances, formed by proteolysis, which activate the amylases, and increase the reaction rate of saccharification.

All three types of diastatic activity are important factors in bread making. Malts and malt extracts used by brewers, distillers, and bakers are purchased largely on the basis of their saccharifying power. A few selected methods for determining saccharogenic, dextrinizing, and saccharifying power are presented. Owing to the historical development of the subject, there is some confusion in terminology. For example, the saccharifying activity of diastases is still very generally referred to as " diastatic " activity, and the latter term is also sometimes used to denote dextrinizing activity.

[248] For fuller discussions see Bailey and Sherwood, *Ind. Eng. Chem.*, 27, 1426 (1935); Gore, *Ind. Eng. Chem.*, 28, 86 (1936); Lüers, *Cereal Chem.*, 13, 153 (1936).

A. DETERMINATION OF THE SACCHAROGENIC POWER OF FLOUR

Method of Blish and Sandstedt. The Association of Official Agricultural Chemists has adopted officially for this purpose the method of Rumsey,[249] further developed by Blish and Sandstedt.[250] The maltose formed is determined not by copper reduction, but with an alkaline ferricyanide solution, as proposed by Hagedorn and Jensen (p. 872). The method[251] is as follows:

Reagents. (*a*) Buffer solution. Make up 3 ml. of glacial acetic acid and 4.1 g. of anhydrous sodium acetate to 1 liter with water. The *p*H of this solution is 4.6–4.8.

(*b*) Alkaline ferricyanide solution. Dissolve 16.5 g. of pure dry potassium ferricyanide and 22 g. of anhydrous sodium carbonate in water to 1 liter. The potassium ferricyanide solution is 0.05 *N*. It maintains its strength for a long period of time if kept in a dark glass bottle away from the light. (The best C.P. grade of this salt purchased on the market may ordinarily be depended upon to be free from moisture and impurities.)

(*c*) Sodium thiosulfate solution, 0.05 *N*. This contains 12.41 g. sodium thiosulfate per liter. Select only the clear crystals from the best C.P. grade. If redistilled water free from carbon dioxide (the second distillation being made after the addition of a small quantity of alkaline potassium permanganate solution to the first distillate, to destroy all traces of organic matter) is used in making up this solution, it will retain its normality for a long time, whereas with ordinary distilled water it is likely to deteriorate slowly on standing. Check the ferricyanide solution against the thiosulfate solution as follows: To 10 ml. of the ferricyanide solution add 25 ml. of the acetic acid reagent (*d*) followed by 1 ml. of 50 per cent potassium iodide solution and 2 ml. of soluble starch solution. Titrate with the thiosulfate solution. (It should require exactly 10 ml. of the thiosulfate solution to completely discharge the blue starch-iodine color.) Standardize the thiosulfate solution against pure iodine solution if necessary.

(*d*) Acetic acid reagent. This contains 200 ml. of glacial acetic acid, 70 g. of potassium chloride, and 20 g. zinc sulfate ($ZnSO_4 \cdot 7\ H_2O$) per liter.

(*e*) Potassium iodide solution, 500 g. to 1 liter. Add 1 drop of concentrated sodium hydroxide solution for each 100 ml. of solution to prevent or substantially delay deterioration of the solution (with liberation of iodine) on standing, which will otherwise occur. The solution must be colorless.

(*f*) Soluble starch solution. One gram of soluble starch in 100 ml. of 30 per cent sodium chloride solution. Prepare a suspension of the soluble starch and pour slowly into boiling water. Add salt and make to volume. The solution should be transparent and colorless.

[249] *Am. Inst. Baking, Bull.* 8, 1922.
[250] *J. Assoc. Official Agr. Chem.*, 17, 394 (1934); 18, 566 (1935).
[251] "Methods of Analysis, A.O.A.C.," 5th ed., pp. 225–227, 1940.

Determination. Introduce 5 g. of flour and a teaspoonful of ignited quartz sand into a 100- or 125-ml. Erlenmeyer flask, and mix by rotating the flask. Add 46 ml. of buffer solution, and again mix by rotating the flask until all the flour is in suspension. (The flask and all ingredients should be individually brought to 30° C. before being mixed together.) Digest for 1 hour at 30° C., preferably in an accurately controlled water thermostat, shaking the flask (by rotation) every 15 minutes. At the end of the hour add 2 ml. of sulfuric acid (3.58 ± 0.05 *N*, approximately 1 + 9), and mix thoroughly. Add 2 ml. of 12 per cent sodium tungstate solution, mix, and let stand a minute or two. Filter through paper (No. 4 Whatman or its equivalent), discarding the first 8 or 10 drops, and pipette 5 ml. of the filtered extract into a test tube of approximately 50-ml. capacity (18–20-mm. diameter). Pipette exactly 10 ml. of the ferricyanide solution into the 5 ml. of extract in the test tube, and immerse the test tube in a vigorously boiling water bath; the surface of the liquid in the test tube should be 3–4 cm. below the surface of the boiling water. (The delay between the filtering of the extract and the treatment in the boiling-water bath should not be more than 15–20 minutes. Further delay may cause a slight error due to sucrose hydrolysis in the acid solution.) Allow the test tube to remain in the boiling-water bath for exactly 20 minutes. Cool the test tube and its contents under running water, and pour at once into a 100- or 125-ml. Erlenmeyer flask. Rinse out the test tube with 25 ml. of the acetic acid solution, and add to the contents of the Erlenmeyer flask, with thorough mixing. Then add 1 ml. of the potassium iodide solution followed by 2 ml. of the starch solution, and mix thoroughly. Titrate with 0.05 *N* sodium thiosulfate to the complete disappearance of the blue color (a 10-ml. burette is recommended). Subtract the number of milliliters of 0.05 *N* sodium thiosulfate used in the titration from 10, which gives milliliters of 0.05 *N* ferricyanide reduced to ferrocyanide by the reducing sugars in the flour extract. This value represents a definite quantity of maltose, which may be ascertained by consulting Table CXXXIX. When 5 ml. of flour extract is used, as herein specified, it is necessary merely to multiply the milligrams of maltose by 20 to obtain milligrams of maltose per 10 g. of flour in 1 hour's diastasis. This is the value that is recorded and reported as the measure of the diastatic value of the flour in question.

The foregoing specifications may be used with all ordinary flours whose values for milligrams of maltose produced by 10 g. of flour in 1 hour will seldom, if ever, exceed 350. For material giving higher values, such as products from malted or sprouted grain, use smaller portions of extract, i.e., 1, 2, or 3 ml. instead of 5 ml. In such cases, however, add enough distilled water to make up the difference, and use a different factor for converting results into milligrams of maltose per 10 g. of flour. Thus, when 2 ml. of extract is used, multiply the value obtained from the table by 50 instead of 20. If the material in the test tubes is colorless instead of yellow, after treatment in the boiling-water bath, and gives no blue color upon the addition of potassium iodide, it is apparent that there was more than enough

maltose to reduce all the ferricyanide, and that the determination must be repeated with a smaller quantity of extract.

TABLE CXXXIX

MALTOSE EQUIVALENT OF FERRICYANIDE SOLUTION*

0.05 *N* Ferricyanide Reduced	Maltose Equivalent	0.05 *N* Ferricyanide Reduced	Maltose Equivalent	0.05 *N* Ferricyanide Reduced	Maltose Equivalent	0.05 *N* Ferricyanide Reduced	Maltose Equivalent
ml.	mg.	ml.	mg.	ml.	mg.	ml.	mg.
0.1	0.2	2.6	4.2	5.1	8.3	7.6	12.3
0.2	0.3	2.7	4.4	5.2	8.4	7.7	12.5
0.3	0.5	2.8	4.5	5.3	8.6	7.8	12.7
0.4	0.6	2.9	4.7	5.4	8.7	7.9	12.9
0.5	0.8	3.0	4.9	5.5	8.9	8.0	13.0
0.6	1.0	3.1	5.0	5.6	9.1	8.1	13.2
0.7	1.1	3.2	5.2	5.7	9.2	8.2	13.4
0.8	1.3	3.3	5.3	5.8	9.4	8.3	13.5
0.9	1.5	3.4	5.5	5.9	9.6	8.4	13.7
1.0	1.6	3.5	5.7	6.0	9.7	8.5	13.9
1.1	1.8	3.6	5.8	6.1	9.9	8.6	14.0
1.2	1.9	3.7	6.0	6.2	10.0	8.7	14.2
1.3	2.1	3.8	6.2	6.3	10.2	8.8	14.4
1.4	2.3	3.9	6.3	6.4	10.4	8.9	14.6
1.5	2.4	4.0	6.5	6.5	10.5	9.0	14.8
1.6	2.6	4.1	6.6	6.6	10.7	9.1	15.0
1.7	2.8	4.2	6.8	6.7	10.9	9.2	15.2
1.8	2.9	4.3	7.0	6.8	11.0	9.3	15.4
1.9	3.1	4.4	7.1	6.9	11.2	9.4	15.6
2.0	3.2	4.5	7.3	7.0	11.3	9.5	15.9
2.1	3.4	4.6	7.5	7.1	11.5	9.6	16.1
2.2	3.6	4.7	7.6	7.2	11.7	9.7	16.5
2.3	3.7	4.8	7.8	7.3	11.8	9.8	17.0
2.4	3.9	4.9	7.9	7.4	12.0	9.9	....
2.5	4.1	5.0	8.1	7.5	12.2	10.0	....

* Prepared by applying the specified procedure to standard solutions of pure maltose and using all reagents in the quantities and volumes precisely as used for flour extracts.

A blank determination, designed to indicate the quantity of reducing sugar originally present in the flour — the value for which presumably should be deducted from the total maltose value after 1 hour's diastasis — has been generally regarded as an essential step in the estimation of flour diastatic activity. This operation, however, is ordinarily unnecessary when dealing with flour milled from *sound* wheat, because the quantity of reducing sugars originally present as such is so small and so nearly constant that it may be disregarded for all practical purposes. The blank determination may therefore be conveniently omitted in ordinary routine testing. It need be used only when there is occasion to doubt the soundness of the wheat, or in cases where there is known to have been an appreciable quantity of frosted, sprouted, heat-damaged, or otherwise unsound kernels in the wheat from which the flour was milled.

To make the blank determination, proceed as follows: Add to 5 g. of flour and a teaspoonful of quartz sand in a 100- or 125-ml. Erlenmeyer flask 48 ml. of 0.4 per cent (by volume) sulfuric acid (preferably pre-cooled to ice-water temperature). Shake the mixture thoroughly again, allow to stand 2 minutes, and filter through a No. 4 Whatman (or its equivalent) paper. Use 5 ml. of the clear filtrate and proceed as before.

In order to increase the range of this method without the necessity of using less than 5 ml. of the flour extract, Sandstedt[252] recommends the use of more concentrated reagents. Tenth normal solutions of the alkaline ferricyanide and of the thiosulfate are prepared, by doubling the weight of the ingredients per liter. The acetic acid-salt mixture contains the same amounts of acetic acid and potassium chloride, but 40 g. of zinc sulfate. The potassium iodide solution and the starch indicator are combined into one: Two grams of soluble starch is suspended in cold water and the mixture is slowly stirred into boiling water; after cooling, 50 g. of potassium iodide is added and the solution is diluted to 100 ml., with the addition of 1 drop of saturated sodium hydroxide solution. The analysis is carried out in the same manner as described above, but the results are found from Table CXL, which gives directly milligrams maltose in 10 g. flour, corresponding to the milliliters $N/10$ thiosulfate used in the titration.

In another modification of the method of Blish and Sandstedt, Hildebrand and McClellan[253] determine the ferrocyanide produced from the ferricyanide by the maltose, with ceric sulfate and Setopaline C as indicator, using the procedure of Miller and Van Slyke (p. 876). With this modification it is possible to measure as much as 35 mg. of maltose in 5 ml. of flour extract (700 mg. maltose in 10 g. flour).

The original method of Blish and Sandstedt and the modification introduced by Hildebrand and McClellan give practically the same results upon flours of medium diastatic activity, significant variations being found only with flours of relatively low or high activity.[254] The Blish and Sandstedt method shows larger experimental errors for low-activity flours, and the Hildebrand and McClellan method for high-activity flours.

Polariscopic measurements may also be employed to follow the saccharogenic degradation of starch, as proposed by Gore.[255] A higher temperature must be used for the 1-hour digestion than in the Blish-Sandstedt method, in order to obtain sufficiently large readings in the

[252] *Cereal Chem.*, 14, 603 (1937).
[253] *Cereal Chem.*, 15, 107 (1938).
[254] Hildebrand, *Cereal Chem.*, 15, 819 (1938).
[255] *J. Assoc. Official Agr. Chem.*, 16, 403 (1933).

TABLE CXL

MALTOSE CONVERSION TABLE FOR 0.1 *N* FERRICYANIDE SOLUTION

0.10 *N* Thiosulfate	Maltose per 10 g. Flour	0.10 *N* Thiosulfate	Maltose per 10 g. Flour	0.10 *N* Thiosulfate	Maltose per 10 g. Flour
ml.	mg.	ml.	mg.	ml.	mg.
0.10	618	3.40	373	6.70	166
0.20	608	3.50	367	6.80	161
0.30	598	3.60	360	6.90	156
0.40	588	3.70	353	7.00	151
0.50	578	3.80	347	7.10	145
0.60	568	3.90	341	7.20	140
0.70	558	4.00	334	7.30	135
0.80	550	4.10	328	7.40	130
0.90	542	4.20	322	7.50	126
1.00	534	4.30	315	7.60	121
1.10	527	4.40	308	7.70	116
1.20	519	4.50	302	7.80	111
1.30	512	4.60	295	7.90	106
1.40	505	4.70	288	8.00	101
1.50	499	4.80	282	8.10	96
1.60	492	4.90	276	8.20	90
1.70	485	5.00	270	8.30	85
1.80	478	5.10	264	8.40	80
1.90	472	5.20	257	8.50	76
2.00	465	5.30	251	8.60	71
2.10	458	5.40	244	8.70	65
2.20	451	5.50	237	8.80	60
2.30	445	5.60	231	8.90	56
2.40	438	5.70	225	9.00	51
2.50	431	5.80	218	9.10	46
2.60	425	5.90	213	9.20	41
2.70	418	6.00	207	9.30	36
2.80	412	6.10	201	9.40	31
2.90	406	6.20	195	9.50	25
3.00	398	6.30	188	9.60	20
3.10	392	6.40	182	9.70	15
3.20	385	6.50	176	9.80	10
3.30	379	6.60	171	9.90	5

polariscope. A blank must be run to correct for the sugars present in the original flour. The results obtained are not directly proportional to those found by the Blish-Sandstedt method, and further study of the method is required.

B. DETERMINATION OF LIQUEFYING AND DEXTRINIZING POWER (α-AMYLASE ACTIVITY)

Determination of Liquefying Power. This is measured, according to Józsa and Gore,[256] by the decrease in viscosity of a starch paste acted upon by a diastatic preparation. A novel way of expressing the

[256] *Ind. Eng. Chem., Anal. Ed.*, **2**, 26 (1930).

activity of the enzyme has been introduced by Johnston and Józsa,[257] who give the following directions for carrying out the determination.[258]

The equipment used consists of:

(1) A high-speed mixer, as used at soda fountains. It is equipped with a Hamilton Beach Universal motor, with vertical shaft, and provided with a silver-plated hexagonal lenticular button, 1 inch at its greatest diameter. The speed without load is about 11,000 r.p.m.

(2) An enameled saucepan about 6 inches wide at the bottom, 9 inches wide at the top, and 5 inches deep.

(3) A 100-mesh brass sieve.

(4) Several precipitating jars of exactly equal dimensions, 2.5 inches wide at the bottom, 2 inches wide at the top, and 5 inches high.

(5) A 15-ml. pipette.

(6) A 100-ml. water-jacketed pipette. The water jacket should contain a thermometer. The pipette should satisfy the specifications of the National Bureau of Standards. The outflow time for water should be 55 to 57 seconds. The delivery tube of the pipette has a mark 2 inches below the bulb. With an 81 per cent glycerol solution of sp. gr. 1.2138 at 20°/20° C., the time of drainage from the upper to the lower mark should be 165 to 190 seconds at 21° C. The tip of the pipette should have a gradual taper of about 2 cm., with a well-finished orifice. Pipettes with longer and especially with irregular tapers should not be used. The inside diameter of the delivery tube is preferably 4.5 mm.

(7) A stop watch recording 0.2 second or less.

(8) One or more accurate thermometers reading to 0.2° C.

(9) Potato starch of the highest obtainable purity. It should be of the B.K.M.F. grade, obtainable from Joseph Morningstar, New York, or of an equally high grade.

Preparation of Standard Starch Paste. Heat to boiling 1800 ml. of distilled water in the enameled, tared saucepan. A heavy glass rod may conveniently be weighed with the saucepan. Weigh out a quantity of the pure potato starch corresponding to 84.22 g. of dry matter. Mix the starch with about 200 ml. of water at 45 to 50° C., stirring vigorously all the time, and as soon as the water in the saucepan begins to boil, pour the mixture into it. Remove the pan from the heater immediately, to prevent burning. Stir the resulting thick mass with the high-speed stirrer for about $1\frac{1}{2}$ minutes. During the mixing the lumps adhering to the sides and bottom of the saucepan are dislodged with the glass rod. Next place the saucepan with the starch paste in a water bath at 20° C., and stir with the glass rod several times to prevent the formation of surface films during cooling. When the mass has cooled to 24–25° C., add 50 ml. of Walpole's acetate buffer, *p*H 4.6 (102 ml. *N* acetic acid and 98 ml. *N* sodium acetate diluted to 1 liter). The resulting *p*H of the mixture is about 5.0 to 5.2. Then make the weight of the starch paste up to

[257] *J. Am. Chem. Soc.*, 57, 701 (1935).

[258] Józsa and Johnston, *Ind. Eng. Chem., Anal. Ed.*, 7, 143 (1935).

2000 g. by the addition of distilled water, stir the paste with the high-speed mixer for 2 minutes to obtain a homogeneous mixture, and pass the paste through the 100-mesh sieve. Only a few small lumps should remain on the sieve.

When the strained paste is mixed with 10 per cent of its weight of a solution containing 250 mg. of sodium chloride in 100 ml., and stirred for 1 minute, it should give the same outflow time at 21° C., within 10 to 15 seconds, as the glycerol solution specified above. For the actual determination, the time of stirring is either shortened or lengthened so that the outflow time checks with that of the glycerol within 2 seconds. This is the initial outflow time.

Besides this initial outflow time, that of a fully liquefied starch paste must also be determined in order to obtain the total possible range of viscosities and the percentage decline in viscosity brought about by the enzyme preparation to be measured.

To liquefy the standard starch paste completely, 900 g. of the strained paste is warmed to 65° C., and 90 g. of a comparatively strong infusion of malt (20 g. of distillers' malt plus 200 ml. of water digested 1 hour at room temperature and filtered) is added. After standing for 1 hour the solution is heated to boiling, cooled, and the original weight (990 g.) is restored by adding water. The viscosity of this fully liquefied starch is measured under the standard conditions specified below, and the result is the final outflow time.

Preparation of the Enzyme Solution. In the case of barley malt, 5 g. is weighed into a 1000-ml. flask, 25 g. of sodium chloride is added, and the flask is filled up to the mark. After standing for 1 hour with occasional shaking, it is filtered, the first 100 ml. of filtrate being discarded. Then 100 ml. of filtrate is transferred to a 1000-ml. flask, and the flask is filled up to the mark.

In case of diastatic malt sirup, 2, 5, 10, or 15 g., according to strength, is weighed out, and 25 g. of sodium chloride is added. Further treatment is the same as described for malt.

The sodium chloride is used because it is an effective desorption or activating agent, and tends to stabilize enzyme infusions. It decreases the viscosity of starch paste, and must therefore be added also in the blank determinations. The temperature of 21° C. was chosen because it has been used for many years in the Lintner method for determining the saccharifying power of malt.

Determination. A 150-g. sample of the standard starch paste is cooled to about 19.5° C., so that, after stirring in the enzyme infusion or the sodium chloride solution, the temperature of the stirred paste is 21° C. ± 0.2°. The degree of precooling will vary with the stirrer and surroundings, but in any case the paste after stirring should be at 21° C. The correct time of stirring for the proper initial outflow time is determined by running one or more blanks. Using this correct time, 15 ml. of enzyme infusion is stirred into 150 g. of paste and the mixture is placed in the bath at 21° C. After 59 minutes the mixture is sucked into the pipette and its outflow time determined. The measurement of the outflow time of the mixture is begun just before the end of the hour reaction period in order to correct for the liquefaction occurring during the measurement. To check the stability of the paste, another blank should be

run on 150 g. of paste which has stood for 1 to 2 hours at 21° C. The outflow time of this blank should not deviate more than 3 or 4 per cent from the first blank. In pipetting the 15-ml. portions it is necessary to avoid the introduction of traces of saliva. A small cotton plug effectively prevents contamination.

Calculation of the Results. The outflow time of the mixture of starch paste and enzyme infusion is deducted from the initial outflow time of the starch paste, and the result is divided into the difference between the initial and the final outflow time. The quotient, multiplied by 100, gives the percentage decline in outflow time, P. From this the milligrams of starch, S, liquefied by the enzyme are calculated by the formula

$$S = 12.9\,P - 0.065\,P^2 + 0.0025\,P^3 \qquad (1)$$

This formula has been derived by Józsa and Johnston from the liquefaction curve of standard starch paste.

The enzyme activity is expressed in "liquefons" per gram of dry enzyme material. This method is based on the fact that, in the case of several enzymes investigated, among them α-amylase, the initial rate of activity on a given substrate is directly proportional to the enzyme concentration. The liquefon is defined as that amount of starch-liquefying enzyme which converts the standard starch paste at the rate of 25 mg. of dry starch per minute at zero time under the conditions specified in the method. Johnston and Józsa found that the logarithm of the number of liquefons is a straight-line function of the milligrams of starch liquefied and that under the experimental conditions chosen the two values are related according to the following equation:

$$\mathrm{Log}_{10}\,L = 0.000565\,(S - 1078) \qquad (2)$$

where L is the number of liquefons per 10 ml. of infusion, and S the milligrams of starch liquefied in 1 hour.

The method of calculation is illustrated by the following example:

	Outflow Time
	seconds
Paste stirred with water for 60 seconds	181.5
Additional stirring 10 seconds (trial)	164.5
New sample stirred with water 65 seconds	171.2
(Outflow time with glycerol solution for this pipette is 170 seconds)	
Initial outflow time	171.2
Final outflow time	56.5
Range in outflow time	114.7
Outflow time with sample (10 mg. per 10 ml.) after 1 hour at 21° C.	91.8
Decline in outflow time, 171.2 − 91.8 =	79.4

Per cent decline 7940 ÷ 114.7 = 69.2.
Milligrams of starch liquefied, formula (1) = 1410.
$\text{Log}_{10} L$, formula (2) = 0.1876; L = 1.540.
The value of 1.540 liquefons is for 10 mg. of the sample; to relate it to 1 g. of sample, L must be multiplied by 100. The liquefon content of the sample is therefore 154 per gram. Since 1 liquefon liquefies 25 mg. of dry starch per minute at zero time, the liquefying power of the sample is 154 × 25, = 3850 mg. starch.

The quantity of enzyme solution to be tested should be adjusted so that a liquefaction corresponding to 50 to 90 per cent decline in viscosity is obtained after 1 hour at 21° C. This is the most suitable range for accurate measurements. Table CXLI gives the concentration of enzyme materials of varying strength for proper application of the method.

TABLE CXLI

ENZYMIC MATERIAL REQUIRED FOR ANALYSIS OF DIFFERENT PREPARATIONS

Liquefons per Gram	Milligrams Enzymic Material per 10 ml. Infusion
1–3	1000
2–6	500
5–15	200
10–30	100
20–60	50
50–150	20
100–300	10
200–600	5
500–1500	2
1000–3000	1

The accuracy of the method is about 5 per cent. The original paper of Józsa and Johnston presents a table giving the values of both S and L for percentage declines from 50 to 90, and obviating the use of the formulas. The method has so far been applied only to malt preparations, the rate curves for takadiastase, pancreatin, etc., not having been determined as yet. If different rate curves are found, this will necessitate merely a change in the empirical formulas.

Method of Wohlgemuth.[259] This method, although differing in principle from that employed by Gore, Józsa, and Johnston, measures a property closely related to the liquefying function of diastases, namely,

[259] *Biochem. Z.*, **9**, 1 (1908).

the rate of dextrinization of soluble starch, the color of the iodine compounds being used as the criterion. It has proved of value in baking technology and may be used advantageously for certain physiological purposes. Several 5-ml. portions of a 1 per cent solution of Lintner's soluble starch (p. 1155) are treated with varying amounts of the diastase preparation at 40° C. for 30 minutes. The solutions are diluted to a definite volume and treated with 1 drop of $N/10$ solution of iodine; after shaking, that tube is selected in which the deep blue or violet of soluble starch has given place to the red or orange red of erythrodextrin. The amount of enzyme added to this tube is noted and its diastatic power calculated as the number of milliliters of 1 per cent soluble-starch solution which would be converted by 1 ml. or by 1 g. of substance. Thus if 0.02 ml. of saliva converts 5 ml. of 1 per cent soluble-starch solution in 30 minutes at 40° C., 1 ml. is supposed to digest 250 ml. The diastatic power of the saliva is then expressed as $D_{30'}^{40^\circ} = 250$ (Wohlgemuth's scale).

The Wohlgemuth method has been criticized by Chesley[260] on the ground that even under the same experimental conditions, and with *p*H control, different samples of soluble starch do not give the same end point. It is therefore necessary to use the same batch of starch or a standardized starch (see p. 1160) for comparative tests.

Method of Ehrnst, Yakish, and Olsen.[261] The end point specified in Wohlgemuth's method is rather indefinite and not readily reproducible. In a modification of it, Ehrnst, Yakish, and Olsen have adopted the end point recommended by Sandstedt, Kneen, and Blish (see below), i.e., the red-brown color produced when a solution of Merck's dextrin ("Reagent" grade) is mixed in definite proportions with iodine solution. The starch conversion is carried out at 30° C. The volumes of soluble-starch solution, of malt infusion, and of iodine solution are kept constant, and the number of minutes necessary to reach the end point is measured with a stop watch. The activity of the malt extract is expressed as the milliliters of 2 per cent soluble-starch solution which would be converted to standard dextrin in 1 hour by 1 ml. of the original malt extract prepared by the standard procedure. A comparison between the results of this method and the method of Józsa and Johnston (p. 1147) showed satisfactory correlation between the quantities of starch converted by proportional amounts of malt.

Method of Sandstedt, Kneen, and Blish for Determining α-Amylase Activity. It has been generally supposed that the Wohlgemuth method gives a measure of α-amylase activity. But Hanes and

[260] *Proc. Soc. Exptl. Biol. Med.*, 31, 1097 (1934).

[261] *Cereal Chem.*, 16, 724 (1939).

Cattle[262] found that the dextrinizing power of α-amylase is increased in the presence of β-amylase. Hence the Wohlgemuth method actually gives the combined effect of these two components of malt diastase. This has been confirmed by Sandstedt, Kneen, and Blish.[263] If it is desired to determine the α-amylase activity itself it is necessary either to exclude β-amylase or to add an excess of β-amylase so as to increase the α-amylase activity to its maximum. Sandstedt, Kneen, and Blish use the latter procedure. The β-amylase solution is prepared by extracting hard winter wheat flour, free from α-amylase, with water at room temperature. The solution is saturated with toluene and kept in a refrigerator. The β-amylase extract is added to a buffered solution of soluble starch from 24 to 48 hours before the test for α-amylase activity is made. During this time the starch is converted to α-amylodextrin which serves as the substrate in the determination. The malt extract or other diastatic material to be tested is added to the amylodextrin solution, the mixture is placed in a water bath kept at 30° C., and aliquots are tested at definite time intervals with iodine solution. The end point is reached when the iodine produces the same red-brown color as with Merck's dextrin ("Reagent" grade). The α-amylase activity is expressed as the number of grams of soluble starch which, in the presence of an excess of β-amylase, are dextrinized by 1 g. of malt in 1 hour at 30° C., under the specified experimental conditions.

For the details of the two methods just described, which have not as yet been tested by other investigators, the chemist is referred to the original articles.

Determination of the "Dextrin Figure" of Flour. In this method, devised by Kent-Jones and Amos,[264] the dextrin formed by the α-amylase in the flour under specified conditions is determined by a modification of the method of Edwards, Nanji, and Chanmugam (see p. 1136).

A 1.25-g. sample of the flour is weighed into a test tube (6 by 1 inch) and is rubbed with 3 ml. of distilled water to a thoroughly smooth paste by means of a rubber-tipped glass rod. The tube is incubated for exactly 30 minutes in a water bath or thermostat heated to 62 ± 0.1° C. It is then placed in cold water and cooled for 4 minutes. The tube must not be agitated or otherwise disturbed from the time it is placed in the hot water until it has cooled. Next 2 ml. of water is added, and the mixture is again rubbed to a smooth paste. This is further diluted with 20 ml. of water, the mixture well stirred, and centrifuged. Ten milliliters of the supernatant liquid is pipetted into a 100-ml. volumetric

[262] *Proc. Roy. Soc. (London)*, **B125**, 387 (1938).
[263] *Cereal Chem.*, **16**, 712 (1939).
[264] *Cereal Chem.*, **17**, 265 (1940).

flask, 2 ml. of 0.1 *N* iodine solution is added, and the volume is completed to the mark with a solution prepared by mixing 50 ml. of water, 50 ml. of 95 per cent alcohol, and 4 ml. of 10 per cent sodium acetate solution. After 5 minutes' standing the starch iodide precipitate is filtered off through a thin layer of purified asbestos, resting on a No. 5 Whatman filter paper in a small Büchner funnel. Then 50 ml. of the clear filtrate is evaporated on a water bath to not less than 5 nor more than 6 ml. The concentrate thus obtained is washed into a 250-ml. beaker with about 10 to 15 ml. of 95 per cent alcohol, and enough alcohol is added to make a total volume of 100 ml. After standing overnight, the precipitated dextrin is filtered off through a tared alundum crucible of medium porosity, washed with alcohol, then with ether, dried for 1 hour at 100° C., and weighed. The result is rounded off to the nearest 0.5 per cent dextrin and reported as the " dextrin figure."

A dextrin figure below 10 indicates that the flour has been milled from sound wheat. If the dextrin figure is above 14, the flour is very apt to cause dampness and stickiness of the crumb after baking. Flours with a dextrin figure between 10 and 14 may be considered suspect, although they may produce satisfactory bread if the baking is done rapidly in a hot oven.

" Diastatic Activity " of Honey; Method of Gothe. Genuine honeys that have not been heated to a high temperature contain varying quantities of diastase, derived principally from the pollen of the flowers visited by the bees. The determination of this diastatic activity, absent in artificial honeys, has been proposed as a criterion for detecting honey adulteration. Gothe[265] introduced for this purpose a method based on the same principle as that of Wohlgemuth. In a modification by Fiehe and Kordatzki,[266] 10 g. of the honey sample is dissolved in 50 to 75 ml. of distilled water, carefully neutralized to phenolphthalein with 0.05 *N* sodium hydroxide, and diluted to 100 ml. In each of a series of ten test tubes are placed 0.5 ml. of 0.1 *N* sodium chloride solution, 0.5 ml. of 0.02 *N* acetic acid, 5 ml. of a 1 per cent soluble-starch solution, and portions of the honey solution ranging from 1 to 10 ml. In two other tubes the same quantities of sodium chloride and acetic acid are placed, then 1 and 2.5 ml. respectively of starch solution, and 10 ml. of honey solution. Enough water is added in each tube to make a total volume of 16 ml. The tubes are all immersed at one time in a water bath heated to 45–50° C., and left there for exactly 1 hour. They are then immediately cooled in ice water, and a drop of 0.1 *N* iodine solution is added to each tube.

[265] *Z. Untersuch. Nahr. u. Genussm.*, 28, 286 (1914).
[266] *Z. Untersuch. Lebensm.*, 55, 162 (1928).

The first tube is noted in which the color has changed from blue or violet to purple, and the diastatic value of the honey is expressed as the number of milliliters of 1 per cent starch solution converted by the diastase present in 1 g. of honey. According to the German food regulations, honey to be accepted as genuine must have a diastatic value not below 8.3.

Bruhns[267] has recommended that the water bath be kept as closely as possible to 45° C., instead of allowing a range of 5°. He also noticed that some honeys absorb iodine, and that for them it is necessary to add more than 1 drop of iodine solution.

Lothrop and Paine[268] found that, as in all other methods for determining diastatic activity, the *p*H of the mixture has a pronounced effect on the results, and that more reliable figures are obtained if it is maintained at about 5.3, with a variation not greater than 0.03, by the addition of a phosphate buffer. This is especially important when the diastatic activity is either high or low, while in the medium range the effect is not so great. Stoldt[269] recommends the addition of sodium acetate in preparing the honey solution.

Schuette and Pauly[270] have made the same observation as Chesley (p. 1151) that different samples of soluble starch give different end points, even if the solutions are well buffered, and that a standard starch must be used. The quantity of iodine employed also affects the color developed, and in doubtful cases a second drop of iodine must be added.

According to Lothrop and Paine there are genuine American honeys which have not been heated and nevertheless give diastatic values below 8.3 by the Gothe method. Great care must be exercised in the interpretation of the results; other criteria, such as the presence of hydroxymethylfurfural, and the glucose-fructose ratio, must be considered in judging a honey suspected of being adulterated with manufactured invert sugar. It must also be kept in mind that long storage, especially at high temperatures, may destroy diastase. On the other hand, diastase may have been added purposely to increase the diastase value.

C. DETERMINATION OF SACCHARIFYING POWER

Lintner's Method for the Determination of the Saccharifying Power of Malt and Malt Extracts.[271] This determination is usually made by the method of Lintner or a modification of it. The results

[267] *Deut. Zuckerind.*, **58**, 921 (1933).
[268] *Ind. Eng. Chem.*, **23**, 71 (1931).
[269] *Z. Untersuch. Lebensm.*, **67**, 435 (1934).
[270] *Ind. Eng. Chem., Anal. Ed.*, **5**, 53 (1933).
[271] *J. prakt. Chem.*, [2], **34**, 378 (1886).

represent the copper-reducing power produced by the action of a measured volume of the extract upon a solution of soluble starch at 21° C. for 1 hour, and are expressed as Lintner degrees. The method in its original form is carried out as follows:

Soluble-Starch Solution. A solution is made containing 2 g. of soluble starch in 100 ml. The soluble starch is prepared by mixing high-grade potato starch with 7.5 per cent hydrochloric acid and allowing the mixture to stand for 6 days at 17 to 20° C. The excess acid is removed by washing, the starch suspended in water, and the last traces of acid are neutralized by adding a little sodium bicarbonate. The starch is washed again, and dried in a gentle current of warm air.

Procedure. In determining the diastatic power of malt, or flour, 25 g. of the finely ground material is digested with 500 ml. of water at room temperature for 5 hours. The solution is then filtered until perfectly clear.

Ten test tubes are placed in a metal rack and 10 ml. of the soluble-starch solution added to each. To the first tube 0.1 ml. of the filtered malt solution is added, to the second tube 0.2 ml., and so on, the tenth tube receiving 1.0 ml. The tubes are shaken and then placed for 1 hour in a water bath kept at 21° C., 5 ml. of mixed Fehling's solution is then added to each tube, and the rack is placed in a boiling-water bath for 10 minutes. The rack is then removed and, after the precipitates of cuprous oxide have settled, the two tubes are selected in which the copper is all reduced and in which some of it still remains in solution, as is shown by the absence or presence of blue color, or by means of the ferrocyanide test. The amount of malt solution just necessary to reduce the 5 ml. of Fehling's solution is between the amounts added to these two tubes; the corrected amount is then assumed to be midway between these limits, or the value of the second decimal estimated from the depth of blue color in the tube where reduction is incomplete.

A malt is given a diastatic value of 100 on Lintner's scale when 0.1 ml. of the filtered 5 per cent extract just reduces 5 ml. of Fehling's solution under the above conditions. If 0.25 ml. of malt solution were required to reduce the 5 ml. of Fehling's solution then the diastatic power of the malt would be $\frac{0.1 \times 100}{0.25} = 40°$ Lintner. A slight correction remains to be made for the reducing sugars in the malt solution and for any reducing power of the soluble starch. This correction is found by taking 5 ml. of Fehling's solution, 10 ml. of starch solution, and 10 ml. of water and heating to boiling. The malt solution is then added from a burette until the blue color is just discharged. If 7 ml. of malt solution were used then there would be a correction of

$\frac{0.1 \times 100}{7}$ = 1.4° Lintner to be subtracted from the value previously found.

In the case of evaporated malt extracts of high diastatic power a 1 per cent or 0.5 per cent solution of the extract is used, the values thus obtained being multiplied by 5 or 10 to obtain the true degrees Lintner for a 5 per cent solution.

The American Association of Cereal Chemists has adopted this method,[272] with the only difference that the malt is digested with water for 6 instead of 5 hours.

Lintner's original procedure has been variously modified during the course of years, with respect to the concentration of the solutions, the time and temperature for extracting the malt and for digesting the extract with the soluble starch, the control of *p*H, the manner of determining the reducing sugar formed, the method of expressing the results, and other details.[273] In England the digestion temperature of 21° C. has been maintained, but in Central Europe it has been changed to 20° C. In both cases a definite volume of malt extract is added to a definite volume of buffered starch solution, the reducing power being determined in England by the method of Lane and Eynon and the results reported as Lintner degrees, while in Central Europe the maltose is determined by the hypoiodite method (p. 895),[274] and the results expressed as grams maltose produced by 100 g. of moisture-free malt. Similar changes have been embodied in the procedure employed by the American Society of Brewing Chemists and adopted also by the Association of Official Agricultural Chemists.

Method of the Association of Official Agricultural Chemists.[275] This is described as follows:

Wash all glassware with acid cleaning solution, then rinse with ordinary tap water not less than 4 times, and finally rinse with distilled water at least twice. Thoroughly dry the digestion flasks.

Reagents. (*a*) Acetate buffer solution. Dissolve 68 g. of sodium acetate ($CH_3COONa \cdot 3\ H_2O$) in 500 ml. of normal acetic acid and make up to 1 liter with water.

(*b*) Soxhlet's modification of Fehling's solution. Prepare by mixing immediately before use equal volumes of the copper sulfate and alkaline tartrate

[272] "Cereal Laboratory Methods," 3rd ed., p. 62, 1935.

[273] For a description of some of these modified methods see Laufer, "Standardizing Methods of Analysis"; reprint of a series of articles published in the *American Brewer*, 1935.

[274] Caldwell, Doebbeling, and Manian, *Ind. Eng. Chem., Anal. Ed.*, 8, 181 (1936).

[275] "Methods of Analysis, A.O.A.C.," 5th ed., pp. 160–162, 1940.

solutions (p. 746). Check the Fehling's solution from time to time by estimating its oxidizing value against a standard solution of invert sugar according to customary analytical procedure.

(*c*) Starch solution. Have the final concentration represent 2 g. of soluble starch (weighed on a dry basis) in 100 ml. of solution. Use starch of such quality and grade that its solubility will be at least 1 : 50 in hot water. It shall contain no dextrins, less than 0.5 per cent reducing substances calculated as maltose, and have a moisture content of approximately 10–12 per cent. A freshly made 2 per cent solution shall have a *p*H between 4.5 and 4.7 without adjustment by the use of a buffer. Subsequent batches of starch shall, when tested on a malt of approximately 100° Lintner on the dry basis and having other characteristics as specified under the determination of extract in malt, show a variation no greater than ±3° Lintner from the value obtained using the original starch in a parallel determination. Further additional batches of starch when purchased shall be tested in parallel with the starch in use. No variation greater than ±3° Lintner will be permitted. In no case shall a cumulative correction as referred to the original starch approved above amount to more than 5° Lintner.

Macerate the starch with a small amount of cold freshly distilled water sufficient to form a smooth thin paste (not over 5 per cent of final volume). Pour this, with constant stirring, into boiling freshly distilled water representing not less than approximately 75 per cent of the final volume of the starch solution at such a rate that boiling does not cease. Continue boiling for 2 minutes after the thin paste is completely introduced. Quickly add an additional 10 per cent of the final volume of cold freshly distilled water to the beaker and transfer the mixture quantitatively to a glass-stoppered volumetric flask, mix by inverting the flask, wash down the neck of the flask, and cool the whole to 20° before adding the buffer solution. Add 2 ml. of the buffer solution for each 100 ml. of the final volume of starch solution and make up the whole to the mark. Mix again by inverting the flask and keep tightly stoppered at 20° until used.

Determination. Grind separately not over 25.5 g. of malt. Collect the finely ground malt in a mash beaker, carefully brushing in the malt particles remaining in the mill. Without delay, adjust the weight of the contents to 25 g. (±0.05 g.). Transfer quantitatively the 25 g. to the container (capacity about 1 liter) in which the infusion is to be made. Add 500 ml. of freshly distilled water and close the container. Let the infusion stand for 2.5 hours at 20° (±0.2°) and agitate by rotation at 20-minute intervals. Take care that in the agitation of the malt suspension as small a quantity as possible of the grist is left adhering to the inner surface of the flask above the level of the water. (Mixing by inverting the flask is specifically cautioned against. Gentle whirling of the contents without splashing on the sides of the container has been found to give sufficient mixing.) Filter the infusion by transferring the entire charge onto a 30–32-cm. fluted filter (CS and S No. 588) contained in a 175-mm. funnel. Return the first 50 ml. of the filtrate to the filter. Collect the filtrate until 3 hours shall have elapsed from the time the water

and ground malt were first mixed. Prevent evaporation during the filtration period as far as possible by placing a watch glass over the funnel and some suitable cover around the stem of the funnel, resting on the neck of the receiver.

Immediately dilute 20 ml. of the above infusion to 100 ml. at 20°, transfer 10 ml. of this infusion to a 200-ml. volumetric flask, and bring to 20°. If the diastatic power of the malt being examined is 135° Lintner or above, make (or repeat) the determination, using a 250-ml. volumetric flask at this point and 200 ml. of the buffered starch solution, and multiply the diastatic power computed by 1.25. Add 100 ml. of buffered starch solution from a fast-flowing pipette at 20°. Mix the solutions by rotating the flask during the addition. Maintain the " starch-infusion " mixture at 20° (±0.2°) for exactly 30 minutes after addition of the starch solution was begun. Add 10 ml. of 0.5 *N* sodium hydroxide rapidly for each 100 ml. of starch solution and mix the whole thoroughly by whirling the flask. Make to the mark at 20° and mix well.

Boil 10 ml. of the Fehling's solution and 10 ml. of water in a small flask with a narrow neck (200-ml. Erlenmeyer). Add from a burette about $\frac{2}{3}$ of the amount of the above digested starch solution probably required and boil 15–20 seconds, rotating constantly. Remove from the flame. If still decidedly blue, add more solution, boil about 10 seconds, and again observe color. When the blue color has been almost discharged, and after boiling gently for about 2 minutes, add 3 drops of a 1 per cent aqueous methylene blue solution. Continue boiling and add more solution until 0.1 ml. or even 1 drop, upon boiling, discharges the blue color. (It becomes violet-lavender as end point nears.)

Repeat the titration, adding at once almost the whole amount of the digested starch required in the above, and proceed to the end point as directed. Let the amount of the digested starch solution required to reach the end point in this second titration be called *A*. Interrupt the boiling as little as possible after the indicator has been added, so that the flask remains filled with steam, preventing much access of air. Upon cooling the blue color usually returns.

Blank Correction. Prepare a blank by proceeding exactly as previously described, except to add the sodium hydroxide to the malt infusion before adding the starch solution. Add to 10 ml. of the Fehling's solution and 10 ml. of water a volume of this blank equal to the final volume of digested starch solution required in the above determination. Boil and again determine the end point, using the digested starch solution, as previously described. Let the amount of digested starch solution used here be called *B*.

To determine the corrected diastatic power (D.P.) solve the formula $\frac{4000}{A} \times \frac{B}{A}$ = D.P. in which $\frac{4000}{A}$ is the apparent diastatic power, which must be modified by the fraction representing the ratio of the blank titration to the original titration which measures the influence of the starch in the determination. To convert this to " dry basis," divide the figure so found by (100 minus per cent moisture). Report as degrees Lintner (dry basis).

Errors in the Determination of Saccharifying Power. Experience with the method described above has shown that, in spite of the detailed directions given, the results obtained in different laboratories with the same sample of malt often show poor agreement. Sallans and Anderson[276] have made a careful study of the sources of error and have found that the principal factors involved are differences in the soluble starch employed and variations in the procedure for determining the reducing power with Fehling's solution. The standard error for either of these factors, when determinations are made in different laboratories, amounts to ±3 per cent for a malt of 100° Lintner. The next important source of error is variation in the temperature at which the starch is converted; the standard error from this source is ±1.4 per cent. The last-named error can be readily avoided by careful temperature control, but the other two require further consideration.

Reliability of the Determination of Reducing Power. The observation of Sallans and Anderson that the determination of the reducing power of the converted starch is an important source of error has been confirmed by a number of other investigators. Gore and Steele[277] have proposed to replace the copper-reduction method by the ferricyanide method used by Blish and Sandstedt for determining the saccharogenic power of flour. This has been recommended also by Anderson and Sallans,[278] by Laufer, Schwarz, and Laufer,[279] and by Rask.[280] Norris and Carter[281] have suggested a ferricyanide method in which the end point is determined with methylene blue, and Burkhart[282] has obtained satisfactory results with the electrometric ferricyanide method of Shaffer and Williams (p. 876).

The consensus of opinion is that the ferricyanide method is preferable because of greater accuracy and speed than is possible with Fehling's solution. It remains to be seen which of the various ferricyanide methods (pp. 872–877) is most satisfactory. Burkert and Dickson[283] have found close agreement between the method of Anderson and Sallans and that of the Association of Official Agricultural Chemists, but the method of Hildebrand and McClellan (p. 1145) gave erratic results.

[276] *Cereal Chem.*, **14**, 708 (1937).
[277] *Ind. Eng. Chem., Anal. Ed.*, 7, 324 (1935).
[278] *Can. J. Research*, **15**, 70 (1937).
[279] *Am. Brewer*, **71**, No. 6, 25 (1938).
[280] *J. Assoc. Official Agr. Chem.*, **22**, 200 (1939).
[281] *J. Inst. Brewing*, **41**, 167 (1935).
[282] *Cereal Chem.*, **16**, 652 (1939).
[283] *Cereal Chem.*, **16**, 657 (1939).

Standardization of the Soluble Starch. The specifications for the soluble starch, prescribed in the method of the Association of Official Agricultural Chemists, have been criticized by Snider and Coleman[284] as being too severe. They propose the following criteria: The starch should give an opalescent, only faintly cloudy solution of about *p*H 4.6; it should contain not more than 1 per cent reducing substances calculated as maltose, nor more than 10 per cent of erythrodextrin determined colorimetrically with iodine; and it should be low in ash (about 0.1 per cent).

Redfern and Johnston[285] consider the rate of saccharification to be the most reliable index of the suitability of the starch for determinations of diastatic power. The method used by them, which is based on previous work by Hanes, may be briefly described as follows:

Reagents. (1) Sodium thiosulfate solution, adjusted iodimetrically to exactly 0.05 *N* with 0.1 *N* potassium iodate solution (3.5672 g. KIO_3 in 1 liter solution). (2) A 1 per cent starch indicator solution, preserved with a few drops of toluene. (3) Zinc sulfate reagent. Dissolve 50 g. of $ZnSO_4 \cdot 7H_2O$ and 100 g. KCl in 600 ml. of distilled water, add 200 ml. of glacial acetic acid, and dilute to 1 liter. If zinc potassium sulfate crystallizes out upon standing, this does no harm. (4) Alkaline ferricyanide solution. This is prepared in the same way as in the method of Blish and Sandstedt (p. 1142). It is checked by pipetting 25 ml. into a 300-ml. Erlenmeyer flask, adding 25 ml. of the zinc sulfate reagent (3), then 5 ml. of 50 per cent potassium iodide solution, and titrating immediately with the 0.05 *N* thiosulfate. If the solution is not exactly 0.05 *N*, it must be adjusted to that strength. (5) Acetate buffer solution. To 1.10 moles of acetic acid is added sufficient of an approximately 4 *N*, carbonate-free sodium hydroxide solution, so that the mixture diluted to 1 liter has a *p*H of 5.00; about 0.73 mole of sodium hydroxide is necessary. (6) Approximately 2 *N* sodium hydroxide and hydrochloric acid; equal volumes of the two should exactly neutralize each other.

The malt solution is prepared by weighing exactly 5 g. of highly active (about 261° Lintner) malt sirup in an aluminum weighing scoop, transferring to a 500-ml. volumetric flask, and diluting to the mark with water. Malt sirup is preferable to dry malt because it is more convenient for making up a fresh solution each day. The malt sirup is kept in a refrigerator at about 3° C.

A portion of the soluble-starch sample to be tested, equivalent to 20 g. of dry substance, is mixed with a little distilled water and stirred

[284] *Cereal Chem.*, **14, 1** (1937).
[285] *Cereal Chem.*, **15, 328** (1938).

into 750 ml. of boiling distilled water. After boiling 2 to 3 minutes longer, the solution is cooled and transferred to a 1-liter volumetric flask, 50 ml. of acetate buffer is added, and the solution is made to the mark.

Determination of the Rate Curve. All the pipettes used should be carefully calibrated and should be protected with cotton plugs to prevent contamination with saliva. A large water bath kept at 25 ± 0.5° C. is used for the determinations, and all the solutions are adjusted to this temperature before a run is started.

Portions of 50 ml. each of the ferricyanide reagent are measured from an automatic pipette into a series of 300-ml. Erlenmeyers, and the flasks immersed in the thermostat bath.

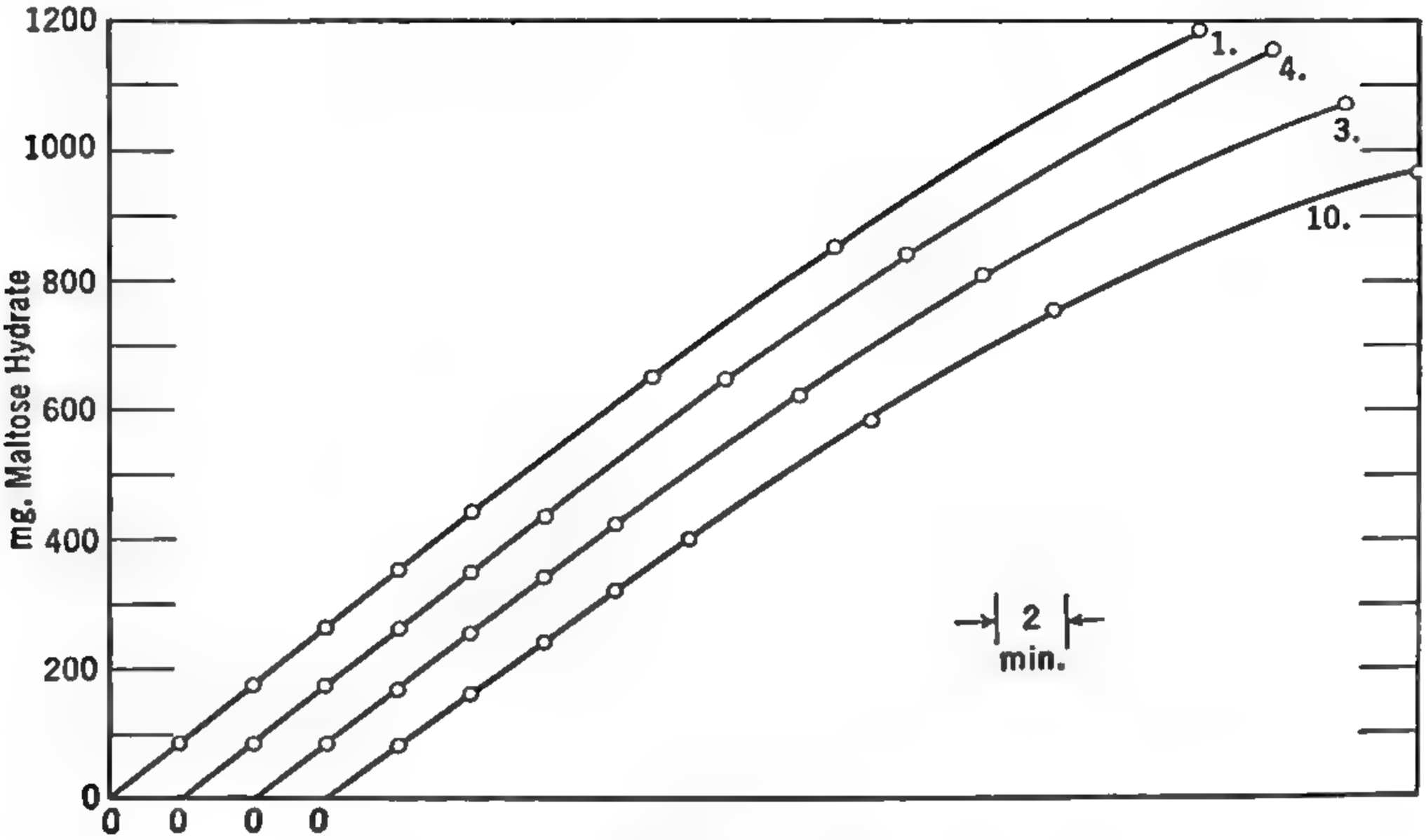
For the conversions, 10 ml. of the malt solution is pipetted into another Erlenmeyer, and after a few minutes 100 ml. of the soluble-starch solution is added from a fast-running pipette. After exactly 2, 4, 6, 8, and 10 minutes from the time the malt and starch solutions were mixed, a 10-ml. portion is delivered into each of five of the flasks containing ferricyanide reagent. After 15, 20, and 30 minutes, 5-ml. portions are similarly transferred. The reducing sugar formed in each of the eight time periods is then determined by diluting the solution in each Erlenmeyer flask to 75 ml., placing the flasks in a boiling-water bath for exactly 20 minutes and then at once in the thermostat kept at 25° C. After cooling for not less than 5 nor more than 60 minutes, 50 ml. of the zinc sulfate reagent and 5 ml. of 50 per cent potassium iodide solution are added, and the liberated iodine is immediately titrated with the 0.05 *N* thiosulfate.

The blank is determined by pipetting 100 ml. of the soluble-starch solution into a 200-ml. volumetric flask and adding 10 ml. of the 2 *N* sodium hydroxide, then 10 ml. of malt solution, and finally 10 ml. of 2 *N* hydrochloric acid. The solution is made to volume, and a 25-ml. aliquot is taken for analysis. The initial reducing power of the starch is determined on a 25-ml. sample of the soluble-starch solution.

The milliliters of 0.05 *N* ferricyanide consumed (*F*) are directly proportional to the milligrams of maltose hydrate oxidized (*M*), according to the equation $M = 1.791\ F$. The amounts of maltose hydrate formed in each interval of time are calculated by means of this equation, and all results are corrected by subtraction of the blank, part of which is due to the malt sirup and part to the starch.

The milligrams of maltose hydrate formed are plotted on a large scale against the time in minutes. The curves are found to be linear for approximately the first 10 minutes, and the initial rate of conversion can be easily determined from this portion of the graph. Some typical

rate curves are shown in Fig. 331, giving the results for four samples of soluble starch.



(*Reproduced with permission from Cereal Chem., 15, 333.*)

FIG. 331. Showing rate curves of the hydrolysis of soluble starch.

The experimental data for these starches were as follows:

No. of Sample	Initial Rate *A*	Maltose Formed in 30 Minutes, *B*	Ratio *B* ÷ *A*
1	44.6	1189	26.7
3	42.9	1078	25.1
4	44.3	1156	26.1
10	41.1	965	23.5

A soluble starch is considered normal if it has an initial rate of 44 to 45 mg. maltose hydrate per minute, and its "saccharification index," i.e., the ratio between the milligrams maltose formed in 30 minutes and the initial rate, is between 26.1 and 26.8. At the same time, the original starch solution should have a low reducing power, preferably not over 2 per cent maltose, calculated on dry starch. Such normal or standard starches will give the same Lintner values within about 2 per cent.

Lintner's Method as Applied to Diastatic Enzymes. In determining the activity of diastase preparations Lintner[286] used the method described for malt, the only difference being that the results are ex-

[286] *J. prakt. Chem.*, [2], 34, 378 (1886); 36, 481 (1888).

pressed in terms of a diastase of which 0.12 mg. produces sufficient sugar to reduce the 5 ml. of Fehling's solution. In making the test, from 50 to 100 mg. of the diastase to be tested is dissolved in 4 to 5 ml. of water and then made up to 100 ml. or 200 ml. according to the supposed strength of the enzyme. If under the conditions described for the malt method 0.2 mg. of a diastase was required to produce sufficient sugar to reduce the 5 ml. of Fehling's solution, then its diastatic power would be $\frac{0.12 \times 100}{0.2} = 60°$ Lintner (diastase scale).

It should be noted that 100° *diastase* is over 40 times $\left(\frac{5.0 \text{ mg.}}{0.12 \text{ mg.}}\right)$ as powerful as 100° *malt* upon Lintner's scale.

Sykes and Mitchell's Gravimetric Modification of Lintner's Method. In the method of Sykes and Mitchell[287] 100 ml. of 2 per cent soluble-starch solution is treated with 1 ml. of the 5 per cent malt extract (prepared as in Lintner's method) at 21° C. for 1 hour; 50 ml. of Fehling's solution is then added and the liquid heated quickly to 98° C., when it is placed in a boiling-water bath for 7 minutes. The reduced copper is then determined, the weight of which divided by 0.438 (the grams of copper in 50 ml. Fehling's solution) and multiplied by 100 gives the diastatic power in degrees of the Lintner scale. The results are said to compare well with those obtained by Lintner's method.

A gravimetric method for determining diastatic power permits a closer degree of estimation than is possible by the original Lintner process. Slight errors of estimation by the volumetric method cause considerable differences in the final results, when only small volumes of diastase solution are taken. Thus between 0.1 ml. and 0.15 ml. the degrees Lintner (malt) will vary between 100 and 66.6.

Gore's Polarimetric Lintner Method.[288] Gore found that the drop in polarization of a mixture of soluble-starch solution with malt extract is directly proportional to the Lintner value calculated from the reducing sugar, and that considerable time may be saved by using the polariscope for the determination. Under the conditions specified below the Lintner degrees may be found by means of the following equation:

$$L = \frac{100\ d}{2.18\ t}$$

where L is Lintner degrees, d the fall in polarization in degrees Ventzke, during time t, measured in hours, and 2.18 the drop in polarization in 1 hour when malt of 100° Lintner is used.

[287] *Analyst*, 21, 122 (1896).
[288] *J. Assoc. Official Agr. Chem.*, 7, 364 (1923/24).

A solution of Lintner's soluble starch, containing 2 g. air-dry starch in 100 ml., is prepared, likewise an infusion of the diastatic product to be tested, of such concentration that 1 ml. contains 50 mg. of sample.

Fifty milliliters of the starch solution is mixed with 0.5 ml. of concentrated ammonia, and 0.5 ml. of the diastase solution, in the order named, and the initial polarization is determined by reading in a 4-dm. tube at 20 to 21° C.

One hundred milliliters of the starch solution is placed in a bath at 21° C., 1 ml. of the diastase solution is added, and after mixing well the solution is allowed to stand for such an interval of time that the fall in polarization does not exceed 3° V. A 50-ml. sample is withdrawn and made alkaline with 0.5 ml. of ammonia; after 25 minutes it is polarized in a 4-dm. tube at 20° to 21° C. The reading is subtracted from the initial reading, and the Lintner degrees are calculated by the formula given above. If the fall in polarization is very small, the remainder of the solution may be digested for a longer period, calculated to result in a total drop of not over 3° V.

The addition of the ammonia serves the double purpose of stopping diastatic action and of hastening the rate of mutarotation of the maltose formed.

If the results are to be expressed in terms of Lintner's diastase scale, instead of the malt scale, the diastase solution is prepared so as to contain 1.2 instead of 50 mg. per ml.

Determination of "Diastatic" Power of Commercial Amylases, Method of Sherman, Kendall and Clark.[289] In studying methods for determining the diastatic power of commercial pancreatin, Sherman, Kendall, and Clark found that the conditions of temperature and activation under which an amylase normally works should be incorporated in the method. These authorities also showed that the amount of reduced copper does not stand in simple proportion to diastatic power, different diastatic values being obtained when different weights of enzyme were taken. These differences are due to the influence of variations in the concentration of starch upon the rate of conversion; if the velocity of the reaction is considered, however, the same diastatic power is derived from the weight of reduced copper for any weight of enzyme. The following gravimetric method was used.

Enzyme. The enzyme may be dissolved in pure water if its power is to be tested immediately. If it is to stand, it should be dissolved in water containing 4 ml. of 0.02 *M* disodium phosphate per 100 ml. The test should be made within an hour in any case. The amount of enzyme to be weighed out will depend entirely on its strength.

[289] *J. Am. Chem. Soc.*, 32, 1073 (1910).

Activating Agents. These will doubtless differ with the different amylases. For pancreatic amylase acting on 2 per cent starch, add 300 mg. sodium chloride and 7 ml. of 0.02 *M* disodium phosphate per 100 ml. (final volume) of reaction mixture.

Procedure. Prepare 400 ml. of 2 per cent soluble-starch solution and the enzyme solution of such a strength that 1 ml. will contain from 0.4 to 1.0 mg. of enzyme. By means of a 1-ml. Mohr's pipette, accurately calibrated in hundredths, measure into four 200-ml. Erlenmeyer flasks such volumes of the solution as will contain 0.2, 0.5, 0.8, and 1.0 mg. of enzyme, respectively. Now 100 ml. of the starch solution, previously warmed to 40° C., is poured into each flask and the digestion allowed to proceed for 30 minutes, the temperature being accurately maintained at 40° C. At the expiration of the 30 minutes, stop the reaction quickly by mixing at once with 50 ml. of Fehling's solution and immerse the flask in a large bath of boiling water for 15 minutes. See that the water of the bath is kept boiling and that it stands above the level of the contents of any of the flasks. At the end of this heating filter quickly and determine the reduced copper by any accurate method.

Correct the weight of reduced copper or cuprous oxide found for the reducing power of the soluble starch by subtracting from it the weight obtained in a "blank" test in which the starch solution is treated directly with the Fehling reagent. Of the four determinations thus corrected, select the highest weight of cuprous oxide which does not exceed 300 mg. and find the corresponding value of *K* in the following table. This value of *K* divided by the milligrams of substance gives the diastatic power of the enzyme upon Sherman's scale.

VALUES FOR *K* FROM CUPROUS OXIDE FOUND

Cuprous Oxide	*K*	Cuprous Oxide	*K*	Cuprous Oxide	*K*	Cuprous Oxide	*K*
mg.		mg.		mg.		mg.	
30	9.1	100	31.2	170	54.1	240	78.3
40	12.2	110	34.4	180	57.5	250	81.8
50	15.3	120	37.6	190	60.9	260	85.4
60	18.4	130	40.9	200	64.3	270	89.0
70	21.6	140	44.2	210	67.8	280	92.6
80	24.8	150	47.5	220	71.3	290	96.3
90	28.0	160	50.8	230	74.8	300	100.0

Example. A sample of soluble starch which had been treated with 1.5 mg. of enzyme gave 295.5 mg. of cuprous oxide; the blank test for the soluble starch gave 60.5 mg. The corrected weight of cuprous oxide is 295.5 − 60.5 = 235 mg. which corresponds to a value for *K* of 76.6; $\frac{76.6}{1.5} = 51$, the diastatic power of the enzyme by Sherman's scale.

The values for K in the above table represent the rate of diastatic conversion and were determined by means of a velocity curve which was plotted with different periods of time as abscissas and different yields of cuprous oxide as ordinates.

Teller[290] has confirmed the conclusion of Sherman, Kendall, and Clark that the conditions under which diastatic preparations are used in practice should be duplicated as closely as possible in the method for determining diastatic power. He likewise claims that certain grains, such as wheat, either ungerminated or germinated, contain two saccharifying enzymes, and that the optimum temperature and pH for their activity are not the same. Determinations made at 21° C., as in the Lintner method, may therefore give misleading results. The best average results for the saccharifying power of the combination of the two enzymes were obtained at 50° C. and a pH of 5.0.

Subsequent researches by Sherman and collaborators, and by others, have shown that the activity of different amylases is affected not only by the hydrogen-ion concentration but also by various salts and by amino acids, and that these factors influence one another. If, for instance, the pH is stabilized with an acetate buffer the results may be different from those when a phosphate buffer is used to give the same pH. In determining diastatic activity it is therefore necessary to adhere strictly to the experimental conditions of the method. On the other hand, the source of the starch used has little effect provided that it has been properly purified and standardized.

As has been pointed out previously, the precision of the original Lintner method leaves much to be desired, especially in the case of highly active malts. Filtration of the cuprous oxide, employed in the methods of Sykes and Mitchell, and of Sherman, Kendall, and Clark, although giving exact results, is too slow for control purposes. The polarimetric method of Gore is rapid and sufficiently accurate but requires the use of a saccharimeter, which is not always available. Among the newer titrimetric methods for the determination of the maltose, the ferricyanide procedure appears to be the most promising as regards both rapidity and precision.

MISCELLANEOUS FOOD PRODUCTS

The detection and estimation of sugars in food products are made according to the physical and chemical methods previously described. Such methods are often valueless, however, for many purposes of the food chemist, who frequently desires to know more about the origin of

[290] *J. Biol. Chem.*, **114, 425 (1936)**; *Cereal Chem.*, **14, 331 (1937)**.

the sugars in his product than about their nature or exact amount. A polarization of maple sugar, for example, will not determine whether its sucrose was derived from the maple or sugar cane. Neither does an estimation of the invert sugar and dextrin in a honey determine whether these have been gathered by the bee or have been added as an adulteration. In the solution of such problems as these the food chemist must base his decision upon reactions and estimations of other ingredients than sugar, such, for example, as the amount of matter precipitated by lead subacetate or by alcohol, the composition of the ash and organic non-sugars, microscopical examination, etc. Such determinations lie strictly outside the province of sugar analysis, and only a few typical applications of such methods will be considered. For a fuller description of such processes the chemist is referred to special works upon food analysis.

Examination of Maple Products

The determination of the amount of lead subacetate precipitate is frequently used as a means of distinguishing pure maple sugars and sirups from those which are adulterated with cane sugar. The method is based upon the presence in maple products, and the absence in cane sugars, of salts of malic acid which gives a copious precipitate with lead subacetate.

Hortvet's[291] Method for Measuring the Volume of Lead Precipitate. *Apparatus.* The apparatus consists of a glass tube and holder as shown in Fig. 332. The tube and holder weigh about 50 g. and should be so constructed that when fitted together the bottom of the tube will be exactly even with the lower surface of the holder. In a set each couple, tube and holder, should be made to balance one another. When placed in the centrifuge there should be as nearly as possible a balanced load carried at the circumference of the wheel.

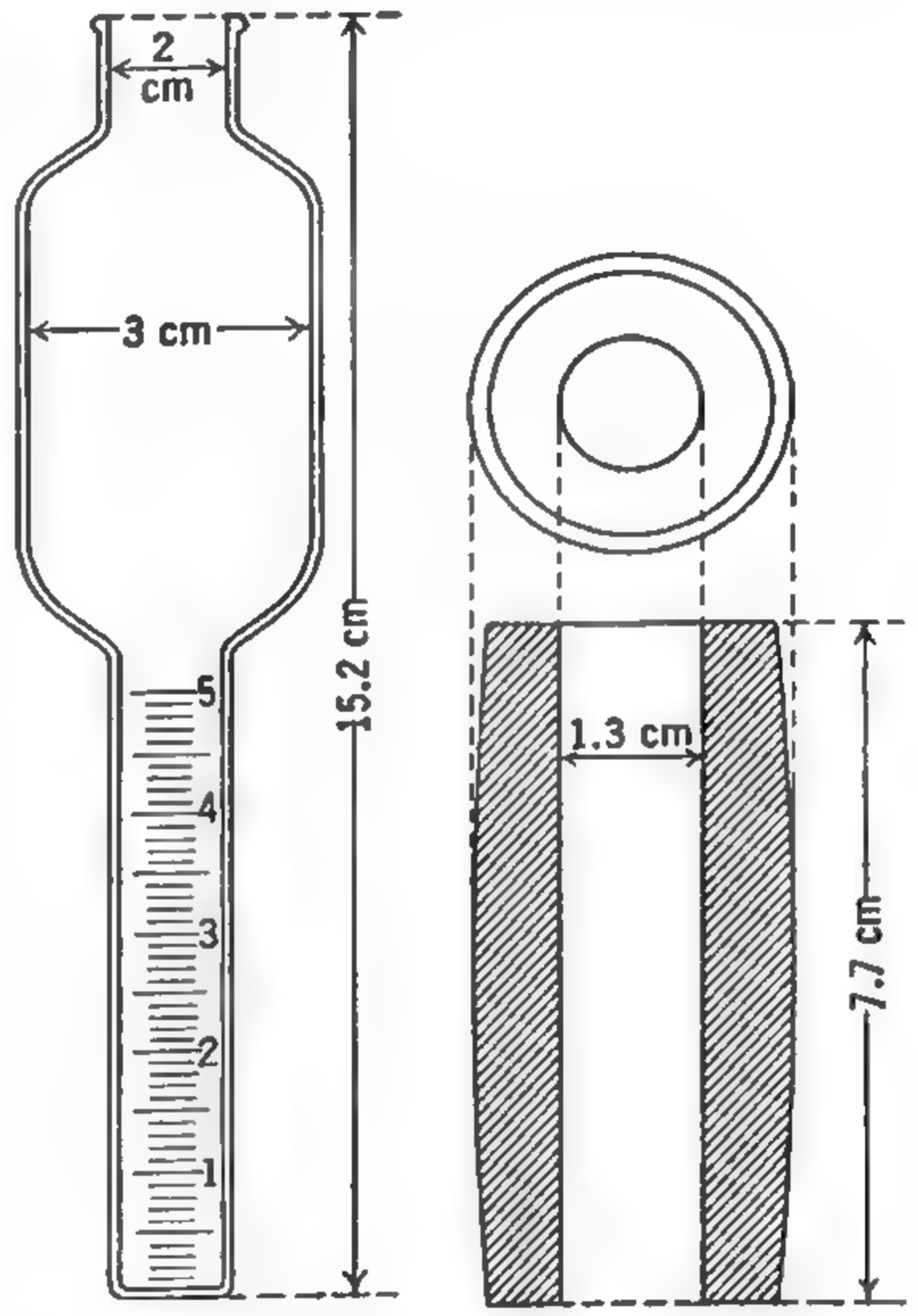


Fig. 332. Hortvet's apparatus for measuring volume of lead precipitate.

[291] *J. Am. Chem. Soc.*, 26, 1523 (1904).

Determination. Introduce into the tube 5 ml. of sirup or 5 g. of sugar, add 10 ml. of water, and dissolve. Add 0.5 ml. (10 drops) of alumina cream (prepared as directed on p. 331) and 1.5 ml. of lead subacetate solution, and shake thoroughly. Allow the mixture to stand from 45 to 60 minutes, occasionally giving the tube a twisting motion to facilitate the settling of the precipitate. Place the tube with its holder in the centrifugal machine and run 6 minutes under the conditions given below. If any material adheres to the sides of the wider portion, remove it by means of a small wire provided with a loop at the end. Return the tube to the centrifuge and run 6 minutes longer at the same rate. Note the volume of the precipitate, estimating to 0.01 ml. as closely as possible. Run a blank, using water and the reagents named above, and correct for same. In the case of a sirup the result is reduced to the 5-g. basis by dividing by the specific gravity of the sample.

The centrifuge used in this method has a radius of 18.5 cm. and is run at a speed of 1600 revolutions per minute. The velocity at the circumference of the wheel is computed in centimeters per second. Calling M (mass) unity in the formula $F = \frac{Mv^2}{r}$, the numerical expression for F, the centrifugal force, becomes 519,363.

By measuring the radius (r) for any given machine and substituting, for F, the numerical constant determination above, the velocity for a given machine may be determined by the following formula: $v = \sqrt{Fr}$. Given the velocity in centimeters per second, the required number of revolutions per second or per minute can be computed.

The volume of lead precipitate, as determined above, was found by Hortvet to vary from 0.94 ml. to 1.82 ml. for pure maple sirups, and from 1.18 ml. to 4.41 ml. for pure maple sugars. Adulterated maple sirups gave from 0.23 ml. to 0.95 ml., and adulterated maple sugars from 0.10 ml. to 1.40 ml.

Winton's[292] Method for Determining Precipitated Lead (Lead Number). Weigh 25 g. of the material, and transfer by means of boiled water into a 100-ml. flask. Add 25 ml. of standard lead subacetate solution, fill to the mark, shake, allow to stand at least 3 hours, and filter through a dry filter. From the clear filtrate pipette off 10 ml., dilute to 50 ml., and add a moderate excess of sulfuric acid (1 ml.) and 100 ml. of 95 per cent alcohol. Let stand overnight, filter on a Gooch crucible, wash with 95 per cent alcohol, dry at a moderate heat, ignite at low redness for 3 minutes, taking care to avoid the reducing cone of the flame, cool and weigh. Calculate the

[292] *J. Am. Chem. Soc.*, 28, 1204 (1906).

amount of lead in the precipitate using the factor 0.68325, subtract this from the amount of lead in 2.5 ml. of the standard solution, multiply the remainder by 100 and divide by 2.5, thus obtaining the lead number.

The standard lead subacetate is prepared by diluting a measured volume of lead subacetate reagent of 1.25 sp. gr. with 4 volumes of water, and filtering if not perfectly clear. The lead in this solution is determined by transferring 25 ml. to a 100-ml. flask, adding a few drops of glacial acetic acid, making up to the mark, shaking, and treating 10 ml. of the solution exactly as described above for the filtrate from the lead precipitate. The addition of acetic acid is necessary to prevent the precipitation of basic salts upon dilution.

The lead number, as determined above, was found by Winton and Kreider to vary from 1.19 to 1.66 for pure maple sirups, and from 1.83 to 2.48 for pure maple sugar. Adulterated maple sirups gave lead numbers ranging from 0.02 to 0.92. Snell and Scott[293] give a range of 0.70 to 2.70 (1.05 to 4.41 on dry basis) for genuine sirups, and Bryan[294] a variation from 1.85 to 4.95 for maple sugars.

The Winton method has been adopted by the Association of Official Agricultural Chemists, with two important changes, one concerning the preparation of the sample and the other that of the lead subacetate solution.

Maple sirups show a large variation in consistency and often contain sugar crystals or insoluble matter. In order to put all samples on a comparable basis, they are prepared as follows for analysis.[295] Any sugar crystals present are dissolved by careful heating. About 100 ml. of the thoroughly mixed sample is transferred to a beaker or casserole, one-quarter of its volume of water is added, and the solution is boiled over a free flame. When the temperature of the boiling sirup approaches 104° C., a small sample is withdrawn with a thin-walled 1-ml. pipette and cooled to room temperature in running water. The outside wall of the pipette is wiped dry, the diluted sirup near the point of the pipette is discarded, and the solids content of the sample is determined with the refractometer. This procedure is repeated from time to time until a reading is obtained corresponding to 64.5 per cent solids, or to such other value as in the experience of the analyst will give a filtered sirup of 65.0 per cent solids. The sirup is then filtered through a filter which will allow the 100 ml. to pass within 5 minutes,

[293] *Ind. Eng. Chem.*, 6, 216 (1914).
[294] *U. S. Dept. Agr. Bull.* 466, 1917.
[295] "Methods of Analysis, A.O.A.C.," 5th ed., p. 512, 1940; *J. Assoc. Official Agr. Chem.*, 16, 79 (1933).

and the filtrate is adjusted to 65.0 ± 0.5 per cent solids by thoroughly mixing with the appropriate quantity of water.

Maple sugars are similarly prepared for analysis by dissolving 100 g. in 150 ml. of water, boiling until the temperature approaches 104° C., and completing the operation as described for sirups.

Snell[296] observed that lead subacetate solutions prepared by the official directions, or by dissolving dry subacetate of lead, varied greatly in composition, particularly in the ratio of basic to neutral lead, and that discrepancies in the lead numbers found by different analysts were largely due to this cause. This source of error is practically eliminated by preparing the lead solution with litharge that has been " activated " by heating to 650–670° C. for 2½ to 3 hours in a muffle. The cooled product should be lemon colored. Eighty grams of normal lead acetate crystals and 40 g. of freshly activated litharge are boiled with 250 ml. water in a 500-ml. Erlenmeyer flask under reflux for 45 minutes. The mixture is cooled, filtered, and diluted with recently boiled water to a specific gravity of 1.25 at 20° C. For the determination of the Winton lead number 1 volume of this solution is diluted with 4 volumes of water, and the diluted solution filtered if necessary.

The Winton lead numbers determined by the revised method range from 1.28 to 3.08 for genuine sirups and average about 32 per cent higher than by the original procedure.

Canadian Lead Number. A procedure somewhat different from that of Winton was devised at about the same time in Canada.[297] According to the revised directions of the Association of Official Agricultural Chemists the Canadian lead number is determined as follows.[298] The lead subacetate solution is prepared from activated litharge as described above, but the solution is used at the specific gravity of 1.25, without further dilution. A quantity of sirup, adjusted to about 65 per cent solids (p. 1169) and containing 25 g. of dry matter (38.46 g. sirup of 65.0 solids by refractometer), is transferred to a 100-ml. flask and made to the mark at 20° C. Twenty milliliters of this solution is pipetted into a large test tube, and 2 ml. of the standard lead subacetate solution is added. The tube is shaken, corked, and allowed to stand for 2 hours. The mixture is filtered with suction through a 25-ml. tared Gooch crucible, having an asbestos mat at least 3 mm. thick. When nearly all the liquid has run through, the crucible is filled with

[296] *J. Assoc. Official Agr. Chem.*, **15**, 181 (1932); **16**, 80 (1933).

[297] *Lab. Inland Revenue Dept. (Ottawa), Bull.* 120, 1906; *Bull.* 140, 1907.

[298] Fowler and Snell, *Ind. Eng. Chem., Anal. Ed.*, 1, 8 (1929); *J. Assoc. Official Agr. Chem.*, 16, 80 (1933); " Methods of Analysis, A.O.A.C.," 5th ed., pp. 513–514, 1940.

cold water. The precipitate is washed in this manner four times, care being taken to prevent the formation of fissures in the precipitate by keeping it covered with water and avoiding too great suction. The precipitate is dried at 100° C. and weighed, and the weight in grams is multiplied by 20.

The range of the Canadian lead number for genuine maple sirups has been reported by Snell as 2.18 to 8.78. When a maple sirup is adulterated with refined cane sugar, the Canadian lead number falls off more rapidly than the maple content, while the Winton lead number falls off less rapidly.

Limitations of the Lead-Precipitate Methods. Raw cane sugars (especially such as are made without clarification and hence contain all the organic salts of the juice) may give amounts of lead precipitate which are as great as those obtained with pure maple products. Doolittle and Seeker[299] give, for example, the following comparison between a Venezuelan muscovado sugar ("Melado") and a pure Vermont maple sugar.

TABLE CXLII

Determination	Muscovado Sugar	Vermont Maple Sugar
Moisture (per cent)	7.50	2.80
Ash (per cent)	1.30	1.10
Polarization, direct at room temperature (°V.)	+82.4	+84.0
Polarization, invert at room temperature (°V.)	−26.8	−29.6
Invert polarization, at 86° (°V.)	± 0.0	± 0.0
Sucrose (Clerget) (per cent)	83.1	85.6
Winton lead number	2.12	2.26

It is seen from the above that the polarization and lead number are not always sufficient to distinguish between cane and maple sugar. The results of the lead-precipitate method should always be confirmed by other means.

Conductivity Value of Maple Products. Adulteration of maple sirup with refined sugar sirup reduces the total ash content. The relative quantity of ionized salts, roughly equivalent to the ash content, may be determined very rapidly by the conductivity method of Snell.[300] For the principle of these measurements and the equipment employed the chemist is referred to Chapter XII, pp. 548–555, and this chapter, pp. 1021–1032. Snell's method has been adopted officially by the Association

[299] *Bull.*, 122, U. S. Bur. Chem., p. 196.

[300] *Trans. Royal Soc. Canada*, 7, III, 165 (1913).

of Official Agricultural Chemists in the following form.[301] Either a flow-through or a dipping cell, with a cell constant of approximately 0.15 cm.$^{-1}$, may be employed. The resistance measurements are made at 25 ± 0.1° C. The cell constant is determined by preparing two solutions of pure, dry potassium chloride, containing 0.3728 and 0.7456 g. respectively, in 500 ml. total volume. The cell is filled with the first (0.01 *M*) solution, the temperature is adjusted to 25° C., the electrical resistance is measured, and the number of ohms found is multiplied by 141.2 (specific conductance × 10^5). The cell is rinsed with the second (0.02 *M*) solution, the measurement repeated, and the number of ohms found is multiplied by 276.1 (specific conductance × 10^5). The two results are averaged..

For the determination, a quantity of sirup containing 25 g. of dry matter is weighed out and transferred to a 100-ml. volumetric flask with warm water of the same quality as used in the determination of the cell constant, the solution is cooled to 25° C. and made to the mark, and the resistance is measured at 25° C. The cell constant is divided by the number of ohms found, and the result represents the conductivity value, expressed as specific conductance × 10^5. For pure maple sirups this figure has been found to vary from 96 to 230, a much smaller range than for the Canadian lead number.

Color Standards for Maple Products. The color of maple sugars and sirups is an important factor in fixing their commercial value. It may be determined by the methods described in Chapter XII, but for trade purposes Bryan[302] proposed to express the color of solid sugars in terms of the Dutch standard (p. 1039). For sirups he suggested a series of color standards, prepared from caramel and glycerol, and numbered 1 to 20. Balch[303] found that the standard caramel made according to Bryan's specifications cannot be readily duplicated, and established a revised series of standards, checked by spectrophotometric determinations. The revised color No. 7 contains 2.75 g. of standard caramel, 32.25 g. of glycerol, and gives in a 1-cm. layer a transmittancy of 68 per cent at a wavelength of 560 mμ; the corrected reading on the Pfund colorimeter (p. 581) is 65.0. The proportions of standard caramel and glycerol, and the transmittancies and Pfund color degrees for the entire color scale, are shown in Table CXLIII.[304]

Any caramel preparation, commercial or made in the laboratory by

[301] *J. Assoc. Official Agr. Chem.*, **16**, 80 (1933); "Methods of Analysis, A.O.A.C.," 5th ed., pp. 514–515, 1940.

[302] *U. S. Dept. Agr., Bur. Chem., Bull.* **134**, 1910.

[303] *Ind. Eng. Chem.*, **22**, 255 (1930).

[304] Balch, private communication.

the usual methods, may be used for preparing the standards. A weighed quantity of caramel is diluted with a weighed quantity of glycerol, and the transmittancy or Pfund color degree is determined. More caramel or glycerol is added to give the desired Bryan color number, and the other color numbers are then made by reference to Table CXLIII. Finally each color standard thus prepared is checked with the spectrophotometer or Pfund colorimeter.

TABLE CXLIII

MAPLE SIRUP COLOR STANDARDS OF BALCH

Bryan Color No.	Caramel	Glycerol	Per Cent Transmittancy at 560 mμ, 1-cm. Layer	Pfund Color Grader, Corrected Readings
	grams	grams		mm.
1	0.00	35.00	100.0	0.0
2	0.25	34.75	96.5	9.0
3	0.50	34.50	92.5	18.5
4	0.88	34.12	87.5	28.5
5	1.40	33.60	81.5	40.0
6	2.00	33.00	75.0	53.0
7	2.75	32.25	68.0	65.0
8	3.50	31.50	60.5	77.0
9	4.40	30.60	52.5	87.0
10	5.60	29.40	44.0	97.0
11	7.00	28.00	35.5	107.0
12	8.75	26.25	27.0	116.0
13	11.00	24.00	19.5	123.5
14	14.00	21.00	13.5	130.5
15	17.00	18.00	9.25	135.5
16	20.00	15.00	6.0	140.0
17	23.50	11.50	4.0	143.3
18	27.0	8.00	2.4	146.0
19	31.0	4.00	1.5	147.8
20	35.0	0.00	0.75	149.5

Determination of Lead in Maple Products. Maple sirups and sugars are frequently found to contain an amount of lead in excess of that permitted by pure food laws. The lead may be derived from the paint used on sap buckets and other containers, from soldered joints or patches on evaporators, or from tin plating containing lead. Methods for its determination have been extensively studied by Wichmann and collaborators,[305] and various procedures have been adopted by the Association of Official Agricultural Chemists for estimating lead in foods.[306] The methods most generally used are based on the formation of a red-

[305] *J. Assoc. Official Agr. Chem.*, 17, 108 (1934); 18, 182 (1935); 19, 130 (1936).

[306] "Methods of Analysis, A.O.A.C.," 5th ed., pp. 394–409, 1940.

colored compound of lead with diphenylthiocarbazone ("dithizone," $C_6H_5NHNHCSN{=}NC_6H_5$), which is soluble in chloroform and other organic solvents. In work of high accuracy, such as in legal borderline cases, it is necessary to ash the product, separate the lead from the dithizone compound electrolytically as the peroxide, and to determine it iodometrically. But this method is very complicated and time consuming. Wichmann has found that in many cases it is sufficient to extract the lead directly from the product without previous ashing and to determine the lead as the dithizone compound photometrically or colorimetrically. A simple and rapid method for maple products, based on the latter procedure, has been devised by Perlman,[307] and is selected for description.

All the chemicals and glassware used should contain a minimum amount of lead. Weigh 15 g. of the maple sirup into a tube or bottle of about 100-ml. capacity which fits into a centrifuge, add 15 ml. of dilute hydrochloric acid (180 ml. concentrated acid in 1 liter of solution), and mix well with the sirup. Dilute with 15 to 25 ml. of water. Add 15 ml. of a reagent prepared by dissolving 20 g. of potassium cyanide and 10 g. of citric acid in 500 ml. of ammonia (28 per cent NH_3), and diluting to 1 liter. This reagent prevents interference by some metals other than lead. Finally add 15 ml. of a solution of 30 mg. of highest-purity dithizone in chloroform, made to a total volume of 1 liter. Shake the entire mixture in the tube vigorously from 100 to 200 times, and centrifuge, to separate into two layers. Introduce a Mohr pipette into the lower layer, placing the finger on the upper end of the pipette until the tip touches the bottom of the tube. Draw a little over 11 ml. of the solution into the pipette, withdraw it, wipe off the tip, and transfer exactly 11 ml. to a 100-ml. separatory funnel in which 11 ml. of dilute hydrochloric acid (see above) has been placed previously. Shake the funnel vigorously 200 times, loosening the upper stopcock at intervals to release the pressure. After the layers have separated sharply, pipette 10 ml. of the aqueous layer into a 50-ml. tall-form Nessler tube, add 10 ml. of the cyanide-citrate reagent, and then 10 ml. of the standard dithizone solution which has been diluted with an equal volume of chloroform, to contain 15 mg. of dithizone per liter. Stopper the Nessler tube, shake vigorously, and compare with the standards.

The stock solution for the standards is made by dissolving 10 g. of pure, dry lead nitrate in 0.1 per cent nitric acid to 1 liter total volume. This is further diluted with the dilute hydrochloric acid so that 1 liter of the standard lead solution contains 3.864 mg. lead. Ten milliliters of this is equivalent to 0.027 grain lead per pound. The colorimetric standards are prepared by measuring into each of ten 50-ml. Nessler tubes from 0 to 10 ml. of the standard lead solution (0 to 0.027 grain

[307] *Ind. Eng. Chem., Anal. Ed.*, 10, 134 (1938).

lead per pound), in steps of 1.11 ml. (0.003 grain), and completing the volume in each tube to 10 ml. by the addition of the dilute hydrochloric acid. Then 10 ml. of the cyanide-citrate reagent and 10 ml. of the dithizone solution containing 15 mg. per liter are added to each tube, which is then vigorously shaken 35 to 50 times. After the layers have separated, the standards are ready for use. They must be made fresh every day and kept in a dark place when not in use.

A comparator block similar to that of Walpole (p. 561), illuminated by a daylight lamp, is used for the determinations. The sample tube is placed in the front center hole, and a tube with chloroform is placed behind it. Behind the two standard tubes on both sides of the sample tube are placed tubes containing chloroform which has been shaken with cyanide-citrate solution and dilute hydrochloric acid in the same manner as the standards. The lead content of the sample is estimated by interpolation between the two nearest standards. A blank determination is run with 15 ml. of water instead of maple sirup, and the blank is deducted from the result obtained upon the sample. If the lead content of the sample is more than 0.027 grain per pound the sirup is first diluted with water and the result multiplied by the dilution factor. Maple sugar is dissolved in water and the resulting sirup used for the determination. Zinc or tin which may be present in the sample does not interfere.

Analysis of Ash as a Means of Determining the Origin of Sugars

One of the most valuable methods of ascertaining the source of a sugar is to determine the composition of its ash. The mineral constituents of the juice of the maple, sugar beet, and sugar cane show very pronounced differences, and, notwithstanding the influences of clarification and crystallization, certain of these constituents find their way into the raw sugar in sufficient quantities to afford a valuable basis of opinion. Sugar-beet juice, for example, in distinction from that of the cane and maple, contains considerable potassium nitrate and perceptible quantities of it are usually present in raw beet sugar. Even the higher grades of beet sugar will frequently respond to delicate tests for nitrates, and this has been used as one means of distinguishing beet from cane sugar.

As an example of the application of the ash-analysis method the following results by Doolittle and Secker[308] upon the muscovado and maple sugar of Table CXLII are given in Table CXLIV. Average de-

[308] *Bull.*, 122, U. S. Bur. Chem., p. 196.

terminations made by Jones[309] upon the ash of pure maple sugars are also added for comparison.

TABLE CXLIV

ANALYSIS OF THE ASH OF MUSCOVADO AND MAPLE SUGAR

Determination	Muscovado Sugar	Vermont Maple Sugar	Average Maple Sugar Ash, by Jones
	per cent	per cent	per cent
Insoluble in boiling nitric acid (1 : 3)	3.41	8.9	.....
Potassium oxide	49.89	23.6	26.49
Sodium oxide	2.32	1.6	.....
Calcium oxide	5.66	35.9	24.98
Magnesium oxide	2.63	3.0	.....
Ferric oxide	0.26	{Slight trace}	.....
Chlorine	1.34	Trace	.....
Sulfur trioxide	23.21	None	1.82
Phosphoric acid	3.68	0.45	.....
Undetermined	7.60	26.55	.....
Water-soluble ash (per cent)	1.23	0.50	0.53
Water-insoluble ash (per cent)	0.17	0.64	0.48
Ratio $\frac{\text{water-soluble ash}}{\text{water-insoluble ash}}$	7.7	0.8	1.1
Alkalinity of water-soluble ash (ml. tenth-normal acid per ash of 1 g. of sample)	0.11	0.49	0.68
Alkalinity of water-insoluble ash (ml. tenth-normal acid per ash of 1 g. of sample)	0.03	1.47	1.01

It is seen that in certain constituents, as potassium oxide, calcium oxide, and sulfur trioxide, the ashes of the muscovado and maple sugars show very pronounced differences. The determinations of water-soluble and water-insoluble ash and of the alkalinities of the latter are valuable aids in forming an opinion as to the origin of a sugar. The ash for such determinations should be prepared according to the method described for quantitative examination (p. 1019).

DETERMINATION OF ALCOHOL PRECIPITATE

The determination of the amount of substance precipitated by strong alcohol is frequently used in examining sugar-containing products. The materials which are precipitated by alcohol may consist of mineral or organic salts, pectin, dextrin, dextran, and other gums. In many cases a qualitative examination of the alcohol precipitate throws considerable light upon the origin of the product.

[309] *Eighteenth Annual Report, Vermont Agr. Exp. Sta.*, p. 331, 1905.

Determination of Alcohol Precipitate in Fruit Products. *Method of the Association of Official Agricultural Chemists.*[310] Sirups or jellies are thoroughly mixed; 300 g. of the sample is weighed into a 2-liter flask and dissolved in water, if necessary by heating on a steam bath. As little heat as possible should be applied, to prevent inversion of sucrose. The solution is cooled, made to the mark, thoroughly mixed by shaking, and filtered if necessary. Fruit juices need only be strained through muslin.

Marmalades, jams, preserves, or fruits are first ground to pulp in a mortar or passed through a food chopper. The pulp is mixed rapidly, to prevent evaporation. A 300-g. portion is boiled in a beaker with 800 ml. of water for 1 hour, the evaporated water being replaced at intervals. The mixture is transferred to a 2-liter flask and further treated as described for sirups or jellies.

One hundred milliliters of the solution obtained is placed in a beaker, 4 to 8 g. of sucrose is added if not already present, and the solution is evaporated to a volume of 20 to 25 ml. If water-insoluble matter separates during the evaporation, more sugar is added. The residual solution is cooled to room temperature and mixed slowly, with constant stirring, with 200 ml. of 95 per cent alcohol. After 1 hour's standing the precipitate is filtered off on a 15-cm. qualitative filter paper and washed with 95 per cent alcohol. The precipitate is washed back quantitatively with hot water from the filter paper into the original beaker. The solution is evaporated down to about 20 ml., and 5 ml. of hydrochloric acid (1 volume concentrated acid plus 2.5 volumes of water) is added. If insoluble matter separates, it is dissolved by stirring and slight warming. The solution is again mixed with 200 ml. of 95 per cent alcohol as before, the mixture allowed to stand for 1 hour, and filtered through paper. The precipitate is thoroughly washed with 95 per cent alcohol to remove all hydrochloric acid. The precipitate is rinsed from the filter paper with hot water into a platinum dish, the solution is evaporated to dryness on a steam bath and then dried to constant weight in a water oven, and the residue is weighed. It is then ignited in a muffle and weighed again. The difference between the dry weight and the ash is the alcohol precipitate.

As the precipitate in many samples is colorless and almost invisible, care must be exercised that none is lost in the dissolving and transferring operations. If the quantity of the alcohol precipitate, as indicated by its volume in the first precipitation, is not excessive, the second filtration may be made through a Gooch crucible containing a thin asbestos mat. If the alcohol precipitate is very pure and small in quantity it

[310] "Methods of Analysis, A.O.A.C.," 5th ed., pp. 335, 340, 1940.

may not be visible at first. In this case, add a small amount of an electrolyte, as sodium chloride, which will flocculate the alcohol precipitate and render it visible.

The ash from the alcohol precipitate should be largely lime and not more than 5 per cent of its total weight. If the ash is larger than this some of the salts of the organic acids have been brought down. Titrate the water-soluble portion of this ash with tenth-normal acid, as any potassium bitartrate precipitated by the alcohol can thus be estimated.

The general appearance of the alcohol precipitate is one of the best indications as to the presence of commercial glucose or dextrin. Upon the addition of alcohol to a pure fruit product a flocculent precipitate is formed with no turbidity, while in the presence of glucose a white turbidity appears at once upon adding the alcohol, and a thick gummy precipitate forms.

When the quantity of gum or dextrin is large, a considerable amount of sugar is sometimes occluded in the alcohol precipitate. This is especially the case with honey, for determining the dextrin in which Browne has modified the alcohol precipitate method as follows.

Determination of Alcohol Precipitate in Honey. *Browne's*[311] *Method.* Eight grams of honey is transferred to a 100-ml. flask with 4 ml. of water and sufficient absolute alcohol to complete to the mark. A little care is required to effect the complete removal of the honey from the weighing dish without using more than 4 ml. of water. The transference is best made by decanting as much as possible of the liquefied honey into the flask, then adding 2 ml. of water to the dish to take up any adhering honey, and again decanting. By using 1 ml. more of the water in two successive washings and adding a few cubic centimeters of the absolute alcohol each time before decanting, the honey can be completely transferred without the necessity of using more water than the 4 ml. Absolute alcohol is used finally to rinse out the dish and is then added to the flask with continual agitation until the volume is completed to 100 ml. After shaking thoroughly the flask is allowed to stand until the dextrin has settled out upon the sides and bottom and the supernatant liquid has become perfectly clear (usually in 24 hours).

The clear solution is then decanted through a filter and the precipitated residue washed with 10 ml. of cold 95 per cent alcohol to remove adhering liquid, the washings being also poured through the filter. The residue adhering to the flask and the particles which may have been caught upon the filter are dissolved in a little boiling distilled water and washed into a weighed platinum dish. The contents of the dish are

[311] *Bull.* 110, U. S. Bur. Chem., p. 19; "Methods of Analysis, A.O.A.C.," 5th ed., p. 510, 1940.

then evaporated and dried in a water oven to constancy in weight. Should the amount of precipitate be considerable, it is necessary to dry upon sand in vacuo at 70° C.

After its weight has been determined, the dried alcohol precipitate is redissolved in water and made to a definite volume. The following dilutions are employed in making up the solutions:

Weight of precipitate in grams	0.0–0.5	0.5–1.0	1.0–1.5	1.5–2.0	2.0–2.5	2.5–3.0
Volume of solution in milliliters	50	100	150	200	250	300

The sugars are then determined in aliquots from the filtered solution of alcohol precipitate both before and after inversion. The total precipitate less ash, invert sugar, and sucrose gives the per cent of dextrin.[312]

Although this method of estimating dextrin in honeys gives much more accurate results than the direct weighing of the alcohol precipitate, it cannot be said in any way to give the true dextrin content of the honey, although it is believed that the figures obtained are a close approximation. A small amount of dextrin always escapes precipitation with alcohol; furthermore, no account is taken of those ingredients which may be occluded in the alcohol precipitate other than the sugars, and no correction is made for the copper-reducing power of the honey dextrin itself. This latter factor, though apparently very small, might prove to be of some importance if much dextrin were present. Notwithstanding these limitations, however, the percentage of dextrin as determined by the method described has been found to have a decided value, especially when it is wished to compare honeys of different origins.

The percentages of dextrin in different American honeys as determined by the above method are given in Table CXLV, which is taken from the work of Browne. The honeys are arranged in order of their dextrin content.

The dextrins of honey are derived largely from *honeydew* (the gummy exudation from leaves, buds, etc.) and not from floral nectar. Honeydew contains considerable mineral matter, and its presence in honey causes a marked increase in the ash content. Honey dextrin is strongly dextrorotatory ($[\alpha]_D$ varies from about +115 to +160), and the presence of much honeydew may cause honey to polarize to the right.

If commercial glucose is suspected, honeydew dextrins may be dis-

[312] With honeydew honey, which gives a large amount of alcohol precipitate, it is found best to take only 4 g. of honey for analysis; in other respects the procedure is the same.

TABLE CXLV

COMPOSITION OF AMERICAN HONEYS. Bull. 110, U. S. Bur. Chem.

Kind of Honey	Number of Samples	Polarization 20° C.	Water	Invert Sugar	Sucrose	Ash	Dextrin	Undetermined
		° V.	per cent	per cent	per cent	per cent	per cent	per cent
Alfalfa	8	−15.10	16.56	76.90	4.42	0.07	0.34	1.71
Apple	2	− 8.55	15.67	73.16	3.69	0.08	0.39	7.01
Orange	1	−15.50	16.99	77.57	0.60	0.08	0.45	4.31
Sweet clover	4	−17.61	17.49	76.20	2.24	0.12	0.45	3.50
Raspberry	2	−18.85	18.08	74.52	1.42	0.05	0.56	5.37
Mangrove	2	−22.80	19.18	76.49	1.73	0.20	0.56	1.84
White clover	15	−13.01	17.64	74.92	1.77	0.07	0.82	4.78
Cotton	2	−17.50	18.35	75.43	1.38	0.21	1.10	3.53
Buckwheat	2	−16.80	18.54	76.85	0.03	0.09	1.22	3.27
Dandelion	2	−12.40	14.54	76.84	3.12	0.16	1.23	4.11
Tupelo	2	−24.00	17.34	72.24	3.01	0.07	2.08	5.26
Golden rod	3	−12.33	19.88	72.02	1.68	0.16	2.18	4.08
Willow	1	−12.80	19.11	71.47	0.95	0.35	2.75	5.37
Basswood	6	− 8.90	17.42	75.14	0.72	0.20	3.07	3.45
Sumac	3	−10.47	18.85	71.11	0.92	0.44	3.57	5.11
Yellow wood	1	− 7.00	18.12	71.51	0.19	0.39	4.10	5.69
White wood	1	− 4.90	17.47	69.02	2.72	0.51	5.59	4.69
Poplar	1	+ 3.60	17.02	65.80	3.10	0.76	10.19	3.13
White oak	1	+11.00	13.56	65.87	4.31	0.79	10.49	4.98
Hickory	1	+ 7.80	16.05	65.89	2.76	0.78	12.95	1.57

tinguished from those of starch conversion by dissolving the alcohol precipitate in a little water and adding a few milliliters of iodine solution; a red color, due to erythrodextrin, indicates the presence of commercial glucose.

According to Raikov,[313] adulteration with commercial glucose may be detected by mixing 1 ml. of glacial acetic acid with 4 to 5 g. of the honey. The dextrins of commercial glucose are precipitated, but not the natural honey dextrins.

DETERMINATION OF PECTIC ACID OR PECTIN

Pectic acid, in the form of its calcium and magnesium salt, is a constituent of the various vegetable complexes that are classified as pectin. A determination of pectic acid not only serves to characterize fruit and other plant products but also is of importance in the preparation of fruit jellies and in the manufacture of sugar from the beet or cane. Several methods have been devised for estimating pectic acid, two of which are described here.

[313] *Z. anal. Chem.*, **116**, 40 (1939).

Method of Wichmann and Chernoff. This method is used by the Association of Official Agricultural Chemists.[314] A solution of the product to be analyzed is prepared in the same way as has been described for the determination of the alcohol precipitate (p. 1177). A 200-ml. aliquot of the solution is transferred to a beaker, 8 to 12 g. of sucrose is added if it is not already present, and the solution is evaporated to about 25 ml. The solution is cooled, and 200 ml. of 95 per cent alcohol is slowly added with constant stirring. The precipitate is allowed to settle, filtered on a 15-cm. qualitative filter paper, and washed with 95 per cent alcohol. It is then transferred with hot water from the filter paper to the original beaker; the solution is evaporated to about 40 ml. and cooled to 25° C. or below. If water-insoluble matter separates during the evaporation, the solution is stirred vigorously, and if necessary it is warmed after the addition of a few drops of dilute hydrochloric acid (1 volume concentrated acid plus 2.5 volumes of water); it is then cooled again. Then from 2 to 5 ml. of 10 per cent sodium hydroxide solution, diluted with water to make a total volume of 50 ml., is added; the volume of the precipitate indicates the amount of sodium hydroxide to be used. After 15 minutes' standing, 40 ml. of water and 10 ml. of dilute hydrochloric acid (1 + 2.5) are added, and the mixture is boiled for 5 minutes. The precipitate of pectic acid is filtered and washed with hot water. This filtration should be rapid, and the filtrate clear. If the filtrate is cloudy or colloidal, the determination is rejected and repeated with more alkali, at a low temperature. The precipitate of pectic acid is washed back from the filter into the original beaker, the volume is adjusted to 40 ml., and the mixture cooled to below 25° C. Then the treatment with dilute sodium hydroxide, precipitation with dilute hydrochloric acid, and boiling is repeated, as described before. The precipitate is filtered and washed with hot water, but only until a test of the filtrate shows a negligible quantity of acid. The filtrate and washings should measure not more than 500 ml. The pectic acid is washed from the filter into a platinum dish, dried on a steam bath, and then to constant weight in a water oven. It is ashed, and weighed again. The loss in weight is pectic acid.

Method of Carré and Haynes.[315] A quantity of solution which will yield from 0.02 to 0.03 g. of calcium pectate is neutralized and diluted to such a volume that after the addition of all the reagents the total amount is about 500 ml. One hundred milliliters of $N/10$ sodium hy-

[314] "Methods of Analysis, A.O.A.C.," 5th ed., pp. 340–341, 1940.

[315] *Biochem. J.*, **16**, 60, 704 (1922); Wichmann, *J. Assoc. Official Agr. Chem.*, **7**, 107 (1923/24).

droxide is added, and the mixture is allowed to stand for at least an hour, but preferably overnight. Then 50 ml. of *N* acetic acid is added, and after 5 minutes 50 ml. of a molar calcium chloride solution. The mixture is allowed to stand for an hour, boiled for a few minutes, and filtered through a large fluted filter. If the precipitation has been properly carried out filtration should be very rapid and subsequent washing easy. The washing is continued with boiling water until the filtrate is free from chloride. The precipitate is washed back into the beaker, boiled, and filtered again. It is then tested for chloride, and these processes are repeated until the filtrate from the boiled precipitate gives no indication of chloride with silver nitrate. It is then filtered through a small fluted filter, from which it is transferred to a dish and finally to a Gooch crucible which has previously been dried at 100° C. The precipitate is dried to constant weight at 100° C. This requires about 12 hours. The result is reported as calcium pectate. This salt contains 7.66 per cent of calcium.

Farnell[316] obtained by a slight modification of this method 1.00 to 1.22 per cent calcium pectate from sugar-cane bagasse. Cane juices contained from 0 to 0.13 per cent on the basis of Brix solids, cane sirup 0.015 per cent, and cane molasses 0.33 to 0.7 per cent.

According to Wichmann[317] the Carré and Haynes method gives high results owing to the difficulty of removing by washing all the calcium chloride adsorbed by the precipitate. He also concluded that the pectic acid in the calcium pectate is different from the pectic acid precipitated by hydrochloric acid, but that it can be transformed into the latter by boiling with 1 per cent hydrochloric acid. If it is then treated with dilute sodium hydroxide and reprecipitated with hydrochloric acid, the pectic acid obtained is the same as that resulting from the direct application of the Wichmann and Chernoff method.

If pectic acid is to be determined in solid materials, such as seeds, hulls, or fibrous plant materials, boiling with water is not sufficient to extract the pectin quantitatively.[318] A solution of ammonium oxalate has been used by some investigators.[319] Other methods prescribe boiling with dilute mineral acids, sometimes preceded by treatment with alkali.

Determination of Pectin. *Method of Schneider and Bock.*[320] These authors have criticized the methods just described on the ground

[316] *Intern. Sugar J.*, **25**, 630 (1923); **26**, 480 (1924).
[317] *J. Assoc. Official Agr. Chem.*, **7**, 107 (1923/24).
[318] *U S. Dept. Agr., Bur. Chem. Bull.* 94, 1905.
[319] Farnell, *Intern. Sugar J.*, **25**, 630 (1923); see also Winkler, *J. Assoc. Official Agr. Chem.*, **20**, 415 (1937); **21**, 440 (1938).
[320] *Angew. Chem.*, **51**, 94 (1938).

that pectic acid is a degradation product of the pectin molecule, and that the results of the determinations are not a reliable measure of the jellying power of the pectin originally present, since the jellying power varies directly as the molecular size. For this reason Schneider and Bock use a much milder treatment to extract the pectin. In this method, designed particularly for the evaluation of the jellying power of commercial pectin preparations, 2 g. of the sample is dissolved by heating with 100 ml. of 0.5 per cent lactic acid on a boiling-water bath for 1 hour. This hydrolyzes the greater part of the pentosans accompanying the pectin. The cooled solution is mixed with 4 volumes of alcohol of such strength that the mixture contains 70 per cent of alcohol. The pectin is precipitated, while the degradation products of lower molecular weight remain in solution. The pectin is filtered off or decanted, redissolved in water and again precipitated with 70 per cent alcohol. It is collected on a Gooch crucible, washed with absolute methyl alcohol, acetone, and ether, and dried to constant weight in a vacuum oven at 69° C. The result is multiplied by 0.95, to correct for the pentosans still present. If desired, the galacturonic acid content of the pectin may be determined by the method of Tollens and Lefèvre (p. 924), and the methoxyl content by the method of Zeisel (p. 945).

BORROWER'S NO.	ISSUE DATE	BORROWER'S NO.	ISSUE DATE

APPENDIX OF SUGAR TABLES

BORROWER'S NO.	ISSUE DATE	BORROWER'S NO.	ISSUE DATE

INTRODUCTION

The following tables, which have been selected to accompany various methods described in the text, have been grouped together for convenience as a separate Appendix, in order to prevent breaking the continuity of the text by the introduction of lengthy tables.

Knowing the very diverse preferences of individual sugar chemists, the authors have made a rather wide selection from the more commonly used copper reduction tables. Limitations of space have obliged them, however, to leave out many tables of recognized merit, and this must be their excuse for any errors of omission.

BORROWER'S NO.	ISSUE DATE	BORROWER'S NO.	ISSUE DATE

TABLE* 1

DENSITY OF AQUEOUS SUCROSE SOLUTIONS AT $\frac{20°}{4°}$ C. (KAISERLICHE NORMAL-EICHUNGS-KOMMISSION.)

Per cent Sucrose	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0	0.998234	0.998622	0.999010	0.999398	0.999786	1.000174	1.000563	1.000952	1.001342	1.001731
1	1.002120	1.002509	1.002897	1.003286	1.003675	1.004064	1.004453	1.004844	1.005234	1.005624
2	1.006015	1.006405	1.006796	1.007188	1.007580	1.007972	1.008363	1.008755	1.009148	1.009541
3	1.009934	1.010327	1.010721	1.011115	1.011510	1.011904	1.012298	1.012694	1.013089	1.013485
4	1.013881	1.014277	1.014673	1.015070	1.015467	1.015864	1.016261	1.016659	1.017058	1.017456
5	1.017854	1.018253	1.018652	1.019052	1.019451	1.019851	1.020251	1.020651	1.021053	1.021454
6	1.021855	1.022257	1.022659	1.023061	1.023463	1.023867	1.024270	1.024673	1.025077	1.025481
7	1.025885	1.026289	1.026694	1.027099	1.027504	1.027910	1.028316	1.028722	1.029128	1.029535
8	1.029942	1.030349	1.030757	1.031165	1.031573	1.031982	1.032391	1.032800	1.033209	1.033619
9	1.034029	1.034439	1.034850	1.035260	1.035671	1.036082	1.036494	1.036906	1.037318	1.037730
10	1.038143	1.038556	1.038970	1.039383	1.039797	1.040212	1.040626	1.041041	1.041456	1.041872
11	1.042288	1.042704	1.043121	1.043537	1.043954	1.044370	1.044788	1.045206	1.045625	1.046043
12	1.046462	1.046881	1.047300	1.047720	1.048140	1.048559	1.048980	1.049401	1.049822	1.050243
13	1.050665	1.051087	1.051510	1.051933	1.052356	1.052778	1.053202	1.053626	1.054050	1.054475
14	1.054900	1.055325	1.055751	1.056176	1.056602	1.057029	1.057455	1.057882	1.058310	1.058737
15	1.059165	1.059593	1.060022	1.060451	1.060880	1.061308	1.061738	1.062168	1.062598	1.063029
16	1.063460	1.063892	1.064324	1.064756	1.065188	1.065621	1.066054	1.066487	1.066921	1.067355
17	1.067789	1.068223	1.068658	1.069093	1.069529	1.069964	1.070400	1.070836	1.071273	1.071710
18	1.072147	1.072585	1.073023	1.073461	1.073900	1.074338	1.074777	1.075217	1.075657	1.076097
19	1.076537	1.076978	1.077419	1.077860	1.078302	1.078744	1.079187	1.079629	1.080072	1.080515
20	1.080959	1.081403	1.081848	1.082292	1.082737	1.083182	1.083628	1.084074	1.084520	1.084967
21	1.085414	1.085861	1.086309	1.086757	1.087205	1.087652	1.088101	1.088550	1.089000	1.089450
22	1.089900	1.090351	1.090802	1.091253	1.091704	1.092155	1.092607	1.093060	1.093513	1.093966
23	1.094420	1.094874	1.095328	1.095782	1.096236	1.096691	1.097147	1.097603	1.098058	1.098514
24	1.098971	1.099428	1.099886	1.100344	1.100802	1.101259	1.101718	1.102177	1.102637	1.103097

* See text, p. 51.

TABLE 1 (*Continued*)

Per cent sucrose	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
25	1.103557	1.104017	1.104478	1.104938	1.105400	1.105862	1.106324	1.106786	1.107248	1.107711
26	1.108175	1.108639	1.109103	1.109568	1.110033	1.110497	1.110963	1.111429	1.111895	1.112361
27	1.112828	1.113295	1.113763	1.114229	1.114697	1.115166	1.115635	1.116104	1.116572	1.117042
28	1.117512	1.117982	1.118453	1.118923	1.119395	1.119867	1.120339	1.120812	1.121284	1.121757
29	1.122231	1.122705	1.123179	1.123653	1.124128	1.124603	1.125079	1.125555	1.126030	1.126507
30	1.126984	1.127461	1.127939	1.128417	1.128896	1.129374	1.129853	1.130332	1.130812	1.131292
31	1.131773	1.132254	1.132735	1.133216	1.133698	1.134180	1.134663	1.135146	1.135628	1.136112
32	1.136596	1.137080	1.137565	1.138049	1.138534	1.139020	1.139506	1.139993	1.140479	1.140966
33	1.141453	1.141941	1.142429	1.142916	1.143405	1.143894	1.144384	1.144874	1.145363	1.145854
34	1.146345	1.146836	1.147328	1.147820	1.148313	1.148805	1.149298	1.149792	1.150286	1.150780
35	1.151275	1.151770	1.152265	1.152760	1.153256	1.153752	1.154249	1.154746	1.155242	1.155740
36	1.156238	1.156736	1.157235	1.157733	1.158233	1.158733	1.159233	1.159733	1.160233	1.160734
37	1.161236	1.161738	1.162240	1.162742	1.163245	1.163748	1.164252	1.164756	1.165259	1.165764
38	1.166269	1.166775	1.167281	1.167786	1.168293	1.168800	1.169307	1.169815	1.170322	1.170831
39	1.171340	1.171849	1.172359	1.172869	1.173379	1.173889	1.174400	1.174911	1.175423	1.175935
40	1.176447	1.176960	1.177473	1.177987	1.178501	1.179014	1.179527	1.180044	1.180560	1.181076
41	1.181592	1.182108	1.182625	1.183142	1.183660	1.184178	1.184696	1.185215	1.185734	1.186253
42	1.186773	1.187293	1.187814	1.188335	1.188856	1.189379	1.189901	1.190423	1.190946	1.191469
43	1.191993	1.192517	1.193041	1.193565	1.194090	1.194616	1.195141	1.195667	1.196193	1.196720
44	1.197247	1.197775	1.198303	1.198832	1.199360	1.199890	1.200420	1.200950	1.201480	1.202010
45	1.202540	1.203071	1.203603	1.204136	1.204668	1.205200	1.205733	1.206266	1.206801	1.207335
46	1.207870	1.208405	1.208940	1.209477	1.210013	1.210549	1.211086	1.211623	1.212162	1.212700
47	1.213238	1.213777	1.214317	1.214856	1.215395	1.215936	1.216476	1.217017	1.217559	1.218101
48	1.218643	1.219185	1.219729	1.220272	1.220815	1.221360	1.221904	1.222449	1.222995	1.223540
49	1.224086	1.224632	1.225180	1.225727	1.226274	1.226823	1.227371	1.227919	1.228469	1.229018

TABLE 1 (*Continued*)

Per cent sucrose	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
50	1.229567	1.230117	1.230668	1.231219	1.231770	1.232322	1.232874	1.233426	1.233979	1.234532
51	1.235085	1.235639	1.236194	1 236748	1.237303	1.237859	1.238414	1.238970	1.239527	1.240084
52	1.240641	1.241198	1.241757	1.242315	1.242873	1.243433	1.243992	1.244552	1.245113	1.245673
53	1.246234	1.246795	1.247358	1.247920	1.248482	1.249046	1.249609	1.250172	1.250737	1.251301
54	1.251866	1.252431	1.252997	1.253563	1.254129	1.254697	1.255264	1.255831	1.256400	1.256967
55	1.257535	1.258104	1.258674	1.259244	1.259815	1.260385	1.260955	1.261527	1.262099	1.262671
56	1.263243	1.263816	1.264390	1.264963	1.265537	1.266112	1.266686	1.267261	1.267837	1.268413
57	1.268989	1.269565	1.270143	1.270720	1.271299	1.271877	1.272455	1.273035	1.273614	1.274194
58	1.274774	1.275354	1.275936	1.276517	1.277098	1.277680	1.278262	1.278844	1.279428	1.280011
59	1.280595	1.281179	1.281764	1.282349	1.282935	1.283521	1.284107	1.284694	1.285281	1.285869
60	1.286456	1.287044	1.287633	1.288222	1.288811	1.289401	1.289991	1.290581	1.291172	1.291763
61	1.292354	1.292946	1.293539	1.294131	1.294725	1.295318	1.295911	1.296506	1.297100	1.297696
62	1.298291	1.298886	1.299483	1.300079	1.300677	1.301274	1.301871	1.302470	1.303068	1.303668
63	1.304267	1.304867	1.305467	1 306068	1.306669	1.307271	1.307872	1.308475	1.309077	1.309680
64	1.310282	1.310885	1.311489	1.312093	1.312699	1.313304	1.313909	1.314515	1.315121	1.315728
65	1.316334	1.316941	1.317549	1.318157	1.318766	1.319374	1.319983	1.320593	1.321203	1.321814
66	1.322425	1.323036	1.323648	1 324259	1.324872	1.325484	1 326097	1.326711	1.327325	1.327940
67	1.328554	1.329170	1.329785	1.330401	1.331017	1.331633	1.332250	1.332868	1.333485	1.334103
68	1 334722	1.335342	1 335961	1.336581	1.337200	1.337821	1.338441	1.339063	1.339684	1.340306
69	1.340928	1.341551	1.342174	1.342798	1.343421	1.344046	1.344671	1.345296	1.345922	1.346547
70	1.347174	1.347801	1.348427	1.349055	1.349682	1.350311	1.350939	1.351568	1.352197	1.352827
71	1.353456	1.354087	1.354717	1.355349	1.355980	1.356612	1.357245	1.357877	1.358511	1.359144
72	1.359778	1.360413	1.361047	1.361682	1.362317	1.362953	1.363590	1.364226	1.364864	1.365501
73	1.366139	1.366777	1.367415	1.368054	1.368693	1.369333	1.369973	1.370613	1.371254	1.371894
74	1.372536	1.373178	1.373820	1.374463	1.375105	1.375749	1.376392	1.377036	1.377680	1.378326

TABLE 1 (*Concluded*)

Per cent Sucrose	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
75	1.378971	1.379617	1.380262	1.380909	1.381555	1.382203	1.382851	1.383499	1.384148	1.384796
76	1.385446	1.386096	1.386745	1.387396	1.388045	1.388696	1.389347	1.389999	1.390651	1.391303
77	1.391956	1.392610	1.393263	1.393917	1.394571	1.395226	1.395881	1.396536	1.397192	1.397848
78	1.398505	1.399162	1.399819	1.400477	1.401134	1.401793	1.402452	1.403111	1.403771	1.404430
79	1.405091	1.405752	1.406412	1.407074	1.407735	1.408398	1.409061	1.409723	1.410387	1.411051
80	1.411715	1.412380	1.413044	1.413709	1.414374	1.415040	1.415706	1.416373	1.417039	1.417707
81	1.418374	1.419043	1.419711	1.420380	1.421049	1.421719	1.422390	1.423059	1.423730	1.424400
82	1.425072	1.425744	1.426416	1.427089	1.427761	1.428435	1.429109	1.429782	1.430457	1.431131
83	1.431807	1.432483	1.433158	1.433835	1.434511	1.435188	1.435866	1.436543	1.437222	1.437900
84	1.438579	1.439259	1.439938	1.440619	1.441299	1.441980	1.442661	1.443342	1.444024	1.444705
85	1.445388	1.446071	1.446754	1.447438	1.448121	1.448806	1.449491	1.450175	1.450860	1.451545
86	1.452232	1.452919	1.453605	1.454292	1.454980	1.455668	1.456357	1.457045	1.457735	1.458424
87	1.459114	1.459805	1.460495	1.461186	1.461877	1.462568	1.463260	1.463953	1.464645	1.465338
88	1.466032	1.466726	1.467420	1.468115	1.468810	1.469504	1.470200	1.470896	1.471592	1.472289
89	1.472986	1.473684	1.474381	1.475080	1.475779	1.476477	1.477176	1.477876	1.478575	1.479275
90	1.479976	1.480677	1.481378	1.482080	1.482782	1.483484	1.484187	1.484890	1.485593	1.486297
91	1.487002	1.487707	1.488411	1.489117	1.489823	1.490528	1.491234	1.491941	1.492647	1.493355
92	1.494063	1.494771	1.495479	1.496188	1.496897	1.497606	1.498316	1.499026	1.499736	1.500447
93	1.501158	1.501870	1.502582	1.503293	1.504006	1.504719	1.505432	1.506146	1.506859	1.507574
94	1.508289	1.509004	1.509720	1.510435	1.511151	1.511868	1.512585	1.513302	1.514019	1.514737
95	1.515455	1.516174	1.516893	1.517612	1.518332	1.519051	1.519771	1.520492	1.521212	1.521934
96	1.522656	1.523378	1.524100	1.524823	1.525546	1.526269	1.526993	1.527717	1.528441	1.529166
97	1.529891	1.530616	1.531342	1.532068	1.532794	1.533521	1.534248	1.534976	1.535704	1.536432
98	1.537161	1.537889	1.538618	1.539347	1.540076	1.540806	1.541536	1.542267	1.542998	1.543730
99	1.544462	1.545194	1.545926	1.546659	1.547392	1.548127	1.548861	1.549595	1.550329	1.551064
100	1.551800									

TABLE* 2

Temperature Corrections for Changing Percentages of Sucrose by Density of Aqueous Solutions to True Values at 20° C.

[This table is calculated using the data on thermal expansion of sugar solutions by Plato, assuming the instrument to be of Jena 16^{III} glass. The table should be used with caution and only for approximate results when the temperature differs much from the standard temperature or from the temperature of the surrounding air.]

Temperature, ° C.	Observed Per Cent of Sugar													
	0	5	10	15	20	25	30	35	40	45	50	55	60	70
	Subtract from Observed Per Cent													
0	0.30	0.49	0.65	0.77	0.89	0.99	1.08	1.16	1.24	1.31	1.37	1.41	1.44	1.49
5	.36	.47	.56	.65	.73	.80	.86	.91	.97	1.01	1.05	1.08	1.10	1.14
10	.32	.38	.43	.48	.52	.57	.60	.64	.67	.70	.72	.74	.75	.77
11	.31	.35	.40	.44	.48	.51	.55	.58	.60	.63	.65	.66	.68	.70
12	.29	.32	.36	.40	.43	.46	.50	.52	.54	.56	.58	.59	.60	.62
13	.26	.29	.32	.35	.38	.41	.44	.46	.48	.49	.51	.52	.53	.55
14	.24	.26	.29	.31	.34	.36	.38	.40	.41	.42	.44	.45	.46	.47
15	.20	.22	.24	.26	.28	.30	.32	.33	.34	.36	.36	.37	.38	.39
16	.17	.18	.20	.22	.23	.25	.26	.27	.28	.28	.29	.30	.31	.32
17	.13	.14	.15	.16	.18	.19	.20	.20	.21	.21	.22	.23	.23	.24
17.5	.11	.12	.12	.14	.15	.16	.16	.17	.17	.18	.18	.19	.19	.20
18	.09	.10	.10	.11	.12	.13	.13	.14	.14	.14	.15	.15	.15	.16
19	.05	.05	.05	.06	.06	.06	.07	.07	.07	.07	.08	.08	.08	.08
	Add to Observed Per Cent													
21	0.04	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.09
22	.10	.10	.11	.12	.12	.13	.14	.14	.15	.15	.16	.16	.16	.16
23	.16	.16	.17	.17	.19	.20	.21	.21	.22	.23	.24	.24	.24	.24
24	.21	.22	.23	.24	.26	.27	.28	.29	.30	.31	.32	.32	.32	.32
25	.27	.28	.30	.31	.32	.34	.35	.36	.38	.38	.39	.39	.40	.39
26	.33	.34	.36	.37	.40	.40	.42	.44	.46	.47	.47	.48	.48	.48
27	.40	.41	.42	.44	.46	.48	.50	.52	.54	.54	.55	.56	.56	.56
28	.46	.47	.49	.51	.54	.56	.58	.60	.61	.62	.63	.64	.64	.64
29	.54	.55	.56	.59	.61	.63	.66	.68	.70	.70	.71	.72	.72	.72
30	.61	.62	.63	.66	.68	.71	.73	.76	.78	.78	.79	.80	.80	.81
35	.99	1.01	1.02	1.06	1.10	1.13	1.16	1.18	1.20	1.21	1.22	1.22	1.23	1.22
40	1.42	1.45	1.47	1.51	1.54	1.57	1.60	1.62	1.64	1.65	1.65	1.65	1.66	1.65
45	1.91	1.94	1.96	2.00	2.03	2.05	2.07	2.09	2.10	2.10	2.10	2.10	2.10	2.08
50	2.46	2.48	2.50	2.53	2.56	2.57	2.58	2.59	2.59	2.58	2.58	2.57	2.56	2.52
55	3.05	3.07	3.09	3.12	3.12	3.12	3.12	3.11	3.10	3.08	3.07	3.05	3.03	2.97
60	3.69	3.72	3.73	3.73	3.72	3.70	3.67	3.65	3.62	3.60	3.57	3.54	3.50	3.43
65	4.4	4.4	4.4	4.4	4.4	4.4	4.3	4.2	4.2	4.1	4.1	4.0	4.0	3.9
70	5.1	5.1	5.1	5.0	5.0	5.0	4.9	4.8	4.8	4.7	4.7	4.6	4.6	4.4
75	6.1	6.0	6.0	5.9	5.8	5.8	5.7	5.6	5.5	5.4	5.4	5.3	5.2	5.0
80	7.1	7.0	7.0	6.9	6.8	6.7	6.6	6.4	6.3	6.2	6.1	6.0	5.9	5.6

* Taken from *Bur. Standards Cir.* 44, 2nd ed., 1918. See also text, p. 52.

The above table may also be used with instruments that are standard at 17.5° C., as follows: Find the correction for reducing to 20° C. in the usual way, and to this add the correction at 17.5° C. with the sign changed; i.e., regarded as positive.

For example, if the instrument reads 20.00 per cent at 24° C., the correction to 17.5° C. is +0.26 +0.15 = 0.41, and the true per cent sugar is 20.41. If it reads 20.00 per cent at 18° C., the correction to 17.5° C. is −0.12 +0.15 = +0.03, and the true per cent sugar is 20.03. If it reads 20.00 at 15° C., the correction is −0.28 +0.15 = −0.13, and the true per cent sugar is 19.87.

TABLE 3*

APPARENT SPECIFIC GRAVITY OF SUCROSE SOLUTIONS AT $\frac{20°}{20°}$ C., WITH CORRESPONDING DEGREES BAUMÉ, AND WEIGHTS PER U. S. GALLON OF SOLUTION

Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.	Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.
0.0	1.00000	0.00	8.322	4.0	1.01569	2.24	8.452
0.1	1.00039	0.06	8.325	4.1	1.01609	2.29	8.456
0.2	1.00078	0.11	8.328	4.2	1.01649	2.35	8.459
0.3	1.00117	0.17	8.331	4.3	1.01689	2.40	8.462
0.4	1.00156	0.22	8.335	4.4	1.01728	2.46	8.465
0.5	1.00195	0.28	8.338	4.5	1.01768	2.52	8.469
0.6	1.00234	0.34	8.341	4.6	1.01808	2.57	8.472
0.7	1.00273	0.39	8.344	4.7	1.01848	2.63	8.475
0.8	1.00312	0.45	8.348	4.8	1.01888	2.68	8.479
0.9	1.00351	0.51	8.351	4.9	1.01928	2.74	8.482
1.0	1.00390	0.56	8.354	5.0	1.01968	2.79	8.485
1.1	1.00429	0.62	8.357	5.1	1.02008	2.85	8.489
1.2	1.00468	0.67	8.361	5.2	1.02048	2.91	8.492
1.3	1.00507	0.73	8.364	5.3	1.02088	2.96	8.495
1.4	1.00546	0.79	8.367	5.4	1.02128	3.02	8.499
1.5	1.00585	0.84	8.370	5.5	1.02168	3.07	8.502
1.6	1.00624	0.90	8.374	5.6	1.02208	3.13	8.505
1.7	1.00663	0.95	8.377	5.7	1.02248	3.18	8.509
1.8	1.00702	1.01	8.380	5.8	1.02289	3.24	8.512
1.9	1.00741	1.07	8.383	5.9	1.02329	3.30	8.515
2.0	1.00780	1.12	8.387	6.0	1.02369	3.35	8.519
2.1	1.00819	1.18	8.390	6.1	1.02409	3.41	8.522
2.2	1.00859	1.23	8.393	6.2	1.02450	3.46	8.526
2.3	1.00898	1.29	8.396	6.3	1.02490	3.52	8.529
2.4	1.00937	1.34	8.400	6.4	1.02530	3.57	8.532
2.5	1.00977	1.40	8.403	6.5	1.02571	3.63	8.536
2.6	1.01016	1.46	8.406	6.6	1.02611	3.69	8.539
2.7	1.01055	1.51	8.409	6.7	1.02652	3.74	8.542
2.8	1.01094	1.57	8.413	6.8	1.02692	3.80	8.546
2.9	1.01134	1.62	8.416	6.9	1.02733	3.85	8.549
3.0	1.01173	1.68	8.419	7.0	1.02773	3.91	8.552
3.1	1.01213	1.74	8.423	7.1	1.02814	3.96	8.556
3.2	1.01253	1.79	8.426	7.2	1.02855	4.02	8.559
3.3	1.01292	1.85	8.429	7.3	1.02895	4.08	8.563
3.4	1.01332	1.90	8.432	7.4	1.02936	4.13	8.566
3.5	1.01371	1.96	8.436	7.5	1.02976	4.19	8.569
3.6	1.01411	2.02	8.439	7.6	1.03017	4.24	8.573
3.7	1.01450	2.07	8.442	7.7	1.03058	4.30	8.576
3.8	1.01490	2.13	8.446	7.8	1.03099	4.35	8.580
3.9	1.01530	2.18	8.449	7.9	1.03139	4.41	8.583

* See text, pp. 51, 66, 82. The apparent specific gravities (in air, brass weights, 20°/20° C.) for 40.0 to 58.9 Brix have been taken from the table of Peters and Phelps (*Bur. Standards Tech. Paper* 338, p. 304); those for 59.0 to 83.9 Brix from that of Brewster and Phelps (*Bur. Standards J. Research*, **10**, 370); those for 0 to 39.9 and from 84.0 to 95.0 Brix have been computed by Zerban and Sattler from Plato's table. The degrees Baumé are taken from *Bur. Standards Tech. Paper* 115. The weights per gallon in air have been interpolated from Table 1 in *Bur. Standards Cir.* 375. All computed figures have been checked against data of the National Bureau of Standards.

TABLE 3 (*Continued*)

Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.	Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.
8.0	1.03180	4.46	8.586	13.0	1.05259	7.24	8.759
8.1	1.03221	4.52	8.590	13.1	1.05301	7.29	8.763
8.2	1.03262	4.58	8.593	13.2	1.05344	7.35	8.766
8.3	1.03303	4.63	8.597	13.3	1.05386	7.40	8.770
8.4	1.03344	4.69	8.600	13.4	1.05429	7.46	8.773
8.5	1.03385	4.74	8.603	13.5	1.05471	7.51	8.777
8.6	1.03426	4.80	8.607	13.6	1.05514	7.57	8.781
8.7	1.03467	4.85	8.610	13.7	1.05556	7.62	8.784
8.8	1.03508	4.91	8.614	13.8	1.05598	7.68	8.788
8.9	1.03549	4.96	8.617	13.9	1.05641	7.73	8.791
9.0	1.03590	5.02	8.620	14.0	1.05683	7.79	8.795
9.1	1.03631	5.07	8.624	14.1	1.05726	7.84	8.798
9.2	1.03673	5.13	8.627	14.2	1.05769	7.90	8.802
9.3	1.03713	5.19	8.631	14.3	1.05812	7.96	8.805
9.4	1.03755	5.24	8.634	14.4	1.05855	8.01	8.809
9.5	1.03796	5.30	8.638	14.5	1.05897	8.07	8.812
9.6	1.03837	5.35	8.641	14.6	1.05940	8.12	8.816
9.7	1.03879	5.41	8.644	14.7	1.05983	8.18	8.820
9.8	1.03920	5.46	8.648	14.8	1.06026	8.23	8.823
9.9	1.03961	5.52	8.651	14.9	1.06068	8.29	8.827
10.0	1.04003	5.57	8.655	15.0	1.06111	8.34	8.830
10.1	1.04044	5.63	8.658	15.1	1.06154	8.40	8.834
10.2	1.04086	5.68	8.662	15.2	1.06197	8.45	8.837
10.3	1.04127	5.74	8.665	15.3	1.06240	8.51	8.841
10.4	1.04169	5.80	8.669	15.4	1.06283	8.56	8.845
10.5	1.04210	5.85	8.672	15.5	1.06327	8.62	8.848
10.6	1.04252	5.91	8.675	15.6	1.06370	8.67	8.852
10.7	1.04293	5.96	8.679	15.7	1.06413	8.73	8.855
10.8	1.04335	6.02	8.682	15.8	1.06456	8.78	8.859
10.9	1.04377	6.07	8.686	15.9	1.06499	8.84	8.863
11.0	1.04418	6.13	8.689	16.0	1.06542	8.89	8.866
11.1	1.04460	6.18	8.693	16.1	1.06585	8.95	8.870
11.2	1.04502	6.24	8.696	16.2	1.06629	9.00	8.873
11.3	1.04544	6.30	8.700	16.3	1.06672	9.06	8.877
11.4	1.04586	6.35	8.703	16.4	1.06716	9.11	8.881
11.5	1.04628	6.41	8.707	16.5	1.06759	9.17	8.884
11.6	1.04670	6.46	8.710	16.6	1.06802	9.22	8.888
11.7	1.04712	6.52	8.714	16.7	1.06846	9.28	8.891
11.8	1.04755	6.57	8.717	16.8	1.06889	9.33	8.895
11.9	1.04796	6.63	8.721	16.9	1.06933	9.39	8 899
12.0	1.04837	6.68	8.724	17.0	1.06976	9.45	8.902
12.1	1.04880	6.74	8.728	17.1	1.07020	9.50	8.906
12.2	1.04922	6.79	8.731	17.2	1.07063	9.56	8.909
12.3	1.04964	6.85	8.735	17.3	1.07107	9.61	8.913
12.4	1.05006	6.90	8.738	17.4	1.07151	9.67	8.917
12.5	1.05048	6.96	8.742	17.5	1.07195	9.72	8.920
12.6	1.05090	7.02	8.745	17.6	1.07238	9.78	8.924
12.7	1.05132	7.07	8.749	17.7	1.07282	9.83	8.928
12.8	1.05174	7.13	8.752	17.8	1.07326	9.89	8.931
12.9	1.05216	7.18	8.756	17.9	1.07370	9.94	8.935

TABLE 3 (*Continued*)

Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.	Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.
18.0	1.07413	10.00	8.939	23.0	1.09647	12.74	9.125
18.1	1.07457	10.05	8.942	23.1	1.09693	12.80	9.128
18.2	1.07501	10.11	8.946	23.2	1.09738	12.85	9.132
18.3	1.07545	10.16	8.950	23.3	1.09784	12.91	9.136
18.4	1.07589	10.22	8.953	23.4	1.09830	12.96	9.140
18.5	1.07633	10.27	8.957	23.5	1.09875	13.02	9.143
18.6	1.07677	10.33	8.961	23.6	1.09921	13.07	9.147
18.7	1.07721	10.38	8.964	23.7	1.09967	13.13	9.151
18.8	1.07765	10.44	8.968	23.8	1.10012	13.18	9.155
18.9	1.07810	10.49	8.972	23.9	1.10058	13.24	9.159
19.0	1.07854	10.55	8.975	24.0	1.10104	13.29	9.163
19.1	1.07898	10.60	8.979	24.1	1.10150	13.35	9.166
19.2	1.07942	10.66	8.983	24.2	1.10196	13.40	9.170
19.3	1.07987	10.71	8.986	24.3	1.10242	13.46	9.174
19.4	1.08031	10.77	8.990	24.4	1.10288	13.51	9.178
19.5	1.08075	10.82	8.994	24.5	1.10334	13.57	9.182
19.6	1.08120	10.88	8.997	24.6	1.10380	13.62	9.185
19.7	1.08164	10.93	9.001	24.7	1.10426	13.67	9.189
19.8	1.08208	10.99	9.005	24.8	1.10472	13.73	9.193
19.9	1.08253	11.04	9.008	24.9	1.10518	13.78	9.197
20.0	1.08297	11.10	9.012	25.0	1.10564	13.84	9.201
20.1	1.08342	11.15	9.016	25.1	1.10610	13.89	9.205
20.2	1.08387	11.21	9.020	25.2	1.10656	13.95	9.208
20.3	1.08431	11.26	9.023	25.3	1.10703	14.00	9.212
20.4	1.08476	11.32	9.027	25.4	1.10749	14.06	9.216
20.5	1.08521	11.37	9.031	25.5	1.10795	14.11	9.220
20.6	1.08565	11.43	9.034	25.6	1.10841	14.17	9.224
20.7	1.08610	11.48	9.038	25.7	1.10888	14.22	9.228
20.8	1.08655	11.54	9.042	25.8	1.10934	14.28	9.232
20.9	1.08699	11.59	9.046	25.9	1.10981	14.33	9.235
21.0	1.08744	11.65	9.049	26.0	1.11027	14.39	9.239
21.1	1.08789	11.70	9.053	26.1	1.11073	14.44	9.243
21.2	1.08834	11.76	9.057	26.2	1.11120	14.49	9.247
21.3	1.08879	11.81	9.061	26.3	1.11167	14.55	9.251
21.4	1.08924	11.87	9.064	26.4	1.11213	14.60	9.255
21.5	1.08969	11.92	9.068	26.5	1.11260	14.66	9.259
21.6	1.09014	11.98	9.072	26.6	1.11307	14.71	9.263
21.7	1.09059	12.03	9.076	26.7	1.11353	14.77	9.266
21.8	1.09104	12.09	9.079	26.8	1.11400	14.82	9.270
21.9	1.09149	12.14	9.083	26.9	1.11447	14.88	9.274
22.0	1.09194	12.20	9.087	27.0	1.11493	14.93	9.278
22.1	1.09239	12.25	9.091	27.1	1.11541	14.99	9.282
22.2	1.09285	12.31	9.094	27.2	1.11587	15.04	9.286
22.3	1.09330	12.36	9.098	27.3	1.11634	15.09	9.290
22.4	1.09375	12.42	9.102	27.4	1.11681	15.15	9.294
22.5	1.09420	12.47	9.106	27.5	1.11728	15.20	9.298
22.6	1.09466	12.52	9.109	27.6	1.11775	15.26	9.302
22.7	1.09511	12.58	9.113	27.7	1.11822	15.31	9.305
22.8	1.09556	12.63	9.117	27.8	1.11869	15.37	9.309
22.9	1.09602	12.69	9.121	27.9	1.11916	15.42	9.313

TABLE 3 (*Continued*)

Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.	Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.
28.0	1.11963	15.48	9.317	33.0	1.14365	18.19	9.517
28.1	1.12011	15.53	9.321	33.1	1.14414	18.25	9.521
28.2	1.12058	15.59	9.325	33.2	1.14463	18.30	9.525
28.3	1.12105	15.64	9.329	33.3	1.14512	18.36	9.529
28.4	1.12153	15.69	9.333	33.4	1.14561	18.41	9.533
28.5	1.12200	15.75	9.337	33.5	1.14610	18.46	9.537
28.6	1.12247	15.80	9.341	33.6	1.14659	18.52	9.541
28.7	1.12295	15.86	9.345	33.7	1.14708	18.57	9.546
28.8	1.12342	15.91	9.349	33.8	1.14757	18.63	9.550
28.9	1.12389	15.97	9.353	33.9	1.14806	18.68	9.554
29.0	1.12437	16.02	9.357	34.0	1.14855	18.73	9.558
29.1	1.12484	16.08	9.361	34.1	1.14905	18.79	9.562
29.2	1.12532	16.13	9.365	34.2	1.14954	18.84	9.566
29.3	1.12580	16.18	9.369	34.3	1.15003	18.90	9.570
29.4	1.12627	16.24	9.372	34.4	1.15053	18.95	9.574
29.5	1.12675	16.29	9.376	34.5	1.15102	19.00	9.578
29.6	1.12723	16.35	9.380	34.6	1.15152	19.06	9.583
29.7	1.12770	16.40	9.384	34.7	1.15201	19.11	9.587
29.8	1.12818	16.46	9.388	34.8	1.15251	19.17	9.591
29.9	1.12866	16.51	9.392	34.9	1.15300	19.22	9.595
30.0	1.12913	16.57	9.396	35.0	1.15350	19.28	9.599
30.1	1.12961	16.62	9.400	35.1	1.15399	19.33	9.603
30.2	1.13009	16.67	9.404	35.2	1.15449	19.38	9.607
30.3	1.13057	16.73	9.408	35.3	1.15499	19.44	9.611
30.4	1.13105	16.78	9.412	35.4	1.15549	19.49	9.616
30.5	1.13153	16.84	9.416	35.5	1.15598	19.55	9.620
30.6	1.13201	16.89	9.420	35.6	1.15648	19.60	9.624
30.7	1.13250	16.95	9.424	35.7	1.15698	19.65	9.628
30.8	1.13298	17.00	9.428	35.8	1.15748	19.71	9.632
30.9	1.13346	17.05	9.432	35.9	1.15797	19.76	9.636
31.0	1.13394	17.11	9.436	36.0	1.15847	19.81	9.640
31.1	1.13442	17.16	9.440	36.1	1.15897	19.87	9.645
31.2	1.13490	17.22	9.444	36.2	1.15948	19.92	9.649
31.3	1.13539	17.27	9.448	36.3	1.15998	19.98	9.653
31.4	1.13587	17.33	9.452	36.4	1.16048	20.03	9.657
31.5	1.13635	17.38	9.456	36.5	1.16098	20.08	9.661
31.6	1.13684	17.43	9.460	36.6	1.16148	20.14	9.665
31.7	1.13732	17.49	9.464	36.7	1.16198	20.19	9.670
31.8	1.13781	17.54	9.468	36.8	1.16248	20.25	9.674
31.9	1.13829	17.60	9.472	36.9	1.16299	20.30	9.678
32.0	1.13877	17.65	9.477	37.0	1.16349	20.35	9.682
32.1	1.13926	17.70	9.481	37.1	1.16399	20.41	9.686
32.2	1.13975	17.76	9.485	37.2	1.16450	20.46	9.691
32.3	1.14023	17.81	9.489	37.3	1.16500	20.52	9.695
32.4	1.14072	17.87	9.493	37.4	1.16551	20.57	9.699
32.5	1.14121	17.92	9.497	37.5	1.16601	20.62	9.703
32.6	1.14170	17.98	9.501	37.6	1.16652	20.68	9.707
32.7	1.14218	18.03	9.505	37.7	1.16702	20.73	9.712
32.8	1.14267	18.08	9.509	37.8	1.16753	20.78	9.716
32.9	1.14316	18.14	9.513	37.9	1.16803	20.84	9.720

TABLE 3 *(Continued)*

Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.	Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.
38.0	1.16853	20.89	9.724	43.0	1.19434	23.57	9.939
38.1	1.16904	20.94	9.728	43.1	1.19486	23.62	9.943
38.2	1.16955	21.00	9.733	43.2	1.19539	23.68	9.948
38.3	1.17006	21.05	9.737	43.3	1.19591	23.73	9.952
38.4	1.17057	21.11	9.741	43.4	1.19644	23.78	9.956
38.5	1.17108	21.16	9.745	43.5	1.19697	23.84	9.961
38.6	1.17159	21.21	9.749	43.6	1.19749	23.89	9.965
38.7	1.17209	21.27	9.754	43.7	1.19802	23.94	9.970
38.8	1.17260	21.32	9.758	43.8	1.19855	24.00	9.974
38.9	1.17311	21.38	9.762	43.9	1.19908	24.05	9.978
39.0	1.17362	21.43	9.766	44.0	1.19961	24.10	9.983
39.1	1.17413	21.48	9.771	44.1	1.20013	24.16	9.987
39.2	1.17465	21.54	9.775	44.2	1.20066	24.21	9.992
39.3	1.17516	21.59	9.779	44.3	1.20119	24.26	9.996
39.4	1.17567	21.64	9.783	44.4	1.20172	24.32	10.000
39.5	1.17618	21.70	9.788	44.5	1.20226	24.37	10.005
39.6	1.17669	21.75	9.792	44.6	1.20279	24.42	10.009
39.7	1.17721	21.80	9.796	44.7	1.20332	24.48	10.014
39.8	1.17772	21.86	9.801	44.8	1.20385	24.53	10.018
39.9	1.17823	21.91	9.805	44.9	1.20438	24.58	10.022
40.0	1.17874	21.97	9.809	45.0	1.20491	24.63	10.027
40.1	1.17926	22.02	9.813	45.1	1.20545	24.69	10.031
40.2	1.17977	22.07	9.818	45.2	1.20598	24.74	10.036
40.3	1.18029	22.13	9.822	45.3	1.20651	24.79	10.040
40.4	1.18080	22.18	9.826	45.4	1.20705	24.85	10.045
40.5	1.18132	22.23	9.831	45.5	1.20758	24.90	10.049
40.6	1.18183	22.29	9.835	45.6	1.20812	24.95	10.054
40.7	1.18235	22.34	9.839	45.7	1.20865	25.01	10.058
40.8	1.18287	22.39	9.843	45.8	1.20919	25.06	10.063
40.9	1.18339	22.45	9.848	45.9	1.20972	25.11	10.067
41.0	1.18390	22.50	9.852	46.0	1.21026	25.17	10.071
41.1	1.18442	22.55	9.856	46.1	1.21080	25.22	10.076
41.2	1.18494	22.61	9.861	46.2	1.21133	25.27	10.080
41.3	1.18546	22.66	9.865	46.3	1.21187	25.32	10.085
41.4	1.18598	22.72	9.869	46.4	1.21241	25.38	10.089
41.5	1.18650	22.77	9.874	46.5	1.21295	25.43	10.094
41.6	1.18702	22.82	9.878	46.6	1.21349	25.48	10.098
41.7	1.18754	22.88	9.882	46.7	1.21402	25.54	10.103
41.8	1.18806	22.93	9.887	46.8	1.21456	25.59	10.107
41.9	1.18858	22.98	9.891	46.9	1.21510	25.64	10.112
42.0	1.18910	23.04	9.895	47.0	1.21564	25.70	10.116
42.1	1.18962	23.09	9.900	47.1	1.21618	25.75	10.121
42.2	1.19014	23.14	9.904	47.2	1.21673	25.80	10.125
42.3	1.19062	23.20	9.908	47.3	1.21727	25.86	10.130
42.4	1.19119	23.25	9.913	47.4	1.21781	25.91	10.134
42.5	1.19171	23.30	9.917	47.5	1.21835	25.96	10.139
42.6	1.19224	23.36	9.921	47.6	1.21889	26.01	10.143
42.7	1.19276	23.41	9.926	47.7	1.21943	26.07	10.148
42.8	1.19329	23.46	9.930	47.8	1.21998	26.12	10.152
42.9	1.19381	23.52	9.935	47.9	1.22052	26.17	10.157

TABLE 3 (*Continued*)

Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.	Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.
48.0	1.22106	26.23	10.161	53.0	1.24874	28.86	10.392
48.1	1.22161	26.28	10.166	53.1	1.24930	28.91	10.396
48.2	1.22215	26.33	10.170	53.2	1.24987	28.96	10.401
48.3	1.22270	26.38	10.175	53.3	1.25043	29.01	10.406
48.4	1.22324	26.44	10.179	53.4	1.25099	29.06	10.410
48.5	1.22379	26.49	10.184	53.5	1.25156	29.12	10.415
48.6	1.22434	26.54	10.189	53.6	1.25212	29.17	10.420
48.7	1.22488	26.59	10.193	53.7	1.25269	29.22	10.425
48.8	1.22543	26.65	10.198	53.8	1.25325	29.27	10.429
48.9	1.22598	26.70	10.202	53.9	1.25382	29.32	10.434
49.0	1.22652	26.75	10.207	54.0	1.25439	29.38	10.439
49.1	1.22707	26.81	10.211	54.1	1.25495	29.43	10.443
49.2	1.22762	26.86	10.216	54.2	1.25552	29.48	10.448
49.3	1.22817	26.91	10.220	54.3	1.25609	29.53	10.453
49.4	1.22872	26.96	10.225	54.4	1.25666	29.59	10.458
49.5	1.22927	27.02	10.230	54.5	1.25723	29.64	10.462
49.6	1.22982	27.07	10.234	54.6	1.25780	29.69	10.467
49.7	1.23037	27.12	10.239	54.7	1.25836	29.74	10.472
49.8	1.23092	27.18	10.243	54.8	1.25893	29.80	10.476
49.9	1.23147	27.23	10.248	54.9	1.25950	29.85	10.481
50.0	1.23202	27.28	10.252	55.0	1.26007	29.90	10.486
50.1	1.23257	27.33	10.257	55.1	1.26064	29.95	10.491
50.2	1.23313	27.39	10.262	55.2	1.26122	30.00	10.495
50.3	1.23368	27.44	10.266	55.3	1.26179	30.06	10.500
50.4	1.23423	27.49	10.271	55.4	1.26236	30.11	10.505
50.5	1.23478	27.54	10.275	55.5	1.26293	30.16	10.510
50.6	1.23534	27.60	10.280	55.6	1.26350	30.21	10.515
50.7	1.23589	27.65	10.285	55.7	1.26408	30.26	10.519
50.8	1.23645	27.70	10.290	55.8	1.26465	30.32	10.524
50.9	1.23700	27.75	10.294	55.9	1.26522	30.37	10.529
51.0	1.23756	27.81	10.299	56.0	1.26580	30.42	10.534
51.1	1.23811	27.86	10.303	56.1	1.26637	30.47	10.538
51.2	1.23867	27.91	10.308	56.2	1.26695	30.52	10.543
51.3	1.23922	27.96	10.312	56.3	1.26752	30.57	10.548
51.4	1.23978	28.02	10.317	56.4	1.26810	30.63	10.553
51.5	1.24034	28.07	10.322	56.5	1.26868	30.68	10.558
51.6	1.24089	28.12	10.326	56.6	1.26925	30.73	10.562
51.7	1.24145	28.17	10.331	56.7	1.26983	30.78	10.567
51.8	1.24201	28.23	10.336	56.8	1.27041	30.83	10.572
51.9	1.24257	28.28	10.340	56.9	1.27098	30.89	10.577
52.0	1.24313	28.33	10.345	57.0	1.27156	30.94	10.581
52.1	1.24369	28.38	10.350	57.1	1.27214	30.99	10.586
52.2	1.24425	28.44	10.354	57.2	1.27272	31.04	10.591
52.3	1.24481	28.49	10.359	57.3	1.27330	31.09	10.596
52.4	1.24537	28.54	10.364	57.4	1.27388	31.15	10.601
52.5	1.24593	28.59	10.368	57.5	1.27446	31.20	10.606
52.6	1.24649	28.65	10.373	57.6	1.27504	31.25	10.610
52.7	1.24705	28.70	10.378	57.7	1.27562	31.30	10.615
52.8	1.24761	28.75	10.382	57.8	1.27620	31.35	10.620
52.9	1.24818	28.80	10.387	57.9	1.27678	31.40	10.625

TABLE 3 (*Continued*)

Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.	Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.
58.0	1.27736	31.46	10.630	63.0	1.30694	34.02	10.876
58.1	1.27794	31.51	10.635	63.1	1.30754	34.07	10.881
58.2	1.27853	31.56	10.640	63.2	1.30815	34.12	10.886
58.3	1.27911	31.61	10.644	63.3	1.30875	34.18	10.891
58.4	1.27969	31.66	10.649	63.4	1.30936	34.23	10.896
58.5	1.28028	31.71	10.654	63.5	1.30994	34.28	10.901
58.6	1.28086	31.76	10.659	63.6	1.31055	34.33	10.906
58.7	1.28145	31.82	10.664	63.7	1.31117	34.38	10.911
58.8	1.28203	31.87	10.669	63.8	1.31177	34.43	10.916
58.9	1.28262	31.92	10.674	63.9	1.31237	34.48	10.921
59.0	1.28320	31.97	10.678	64.0	1.31297	34.53	10.926
59.1	1.28379	32.02	10.683	64.1	1.31359	34.58	10.931
59.2	1.28437	32.07	10.688	64.2	1.31418	34.63	10.936
59.3	1.28497	32.13	10.693	64.3	1.31479	34.68	10.941
59.4	1.28556	32.18	10.698	64.4	1.31540	34.74	10.946
59.5	1.28614	32.23	10.703	64.5	1.31600	34.79	10.951
59.6	1.28672	32.28	10.708	64.6	1.31661	34.84	10.956
59.7	1.28731	32.33	10.713	64.7	1.31723	34.89	10.961
59.8	1.28789	32.38	10.718	64.8	1.31784	34.94	10.967
59.9	1.28849	32.43	10.722	64.9	1.31845	34.99	10.972
60.0	1.28908	32.49	10.727	65.0	1.31905	35.04	10.977
60.1	1.28966	32.54	10.732	65.1	1.31966	35.09	10.982
60.2	1.29025	32.59	10.737	65.2	1.32028	35.14	10.987
60.3	1.29084	32.64	10.742	65.3	1.32089	35.19	10.992
60.4	1.29143	32.69	10.747	65.4	1.32150	35.24	10.997
60.5	1.29203	32.74	10.752	65.5	1.32210	35.29	11.002
60.6	1.29262	32.79	10.757	65.6	1.32271	35.34	11.007
60.7	1.29321	32.85	10.762	65.7	1.32332	35.39	11.012
60.8	1.29380	32.90	10.767	65.8	1.32393	35.45	11.017
60.9	1.29439	32.95	10.772	65.9	1.32455	35.50	11.022
61.0	1.29498	33.00	10.777	66.0	1.32516	35.55	11.027
61.1	1.29559	33.05	10.781	66.1	1.32577	35.60	11.033
61.2	1.29618	33.10	10.786	66.2	1.32638	35.65	11.038
61.3	1.29677	33.15	10.791	66.3	1.32699	35.70	11.043
61.4	1.29736	33.20	10.796	66.4	1.32759	35.75	11.048
61.5	1.29796	33.26	10.801	66.5	1.32820	35.80	11.053
61.6	1.29855	33.31	10.806	66.6	1.32884	35.85	11.058
61.7	1.29915	33.36	10.811	66.7	1.32945	35.90	11.063
61.8	1.29975	33.41	10.816	66.8	1.33007	35.95	11.068
61.9	1.30034	33.46	10.821	66.9	1.33068	36.00	11.073
62.0	1.30093	33.51	10.826	67.0	1.33129	36.05	11.079
62.1	1.30153	33.56	10.831	67.1	1.33192	36.10	11.084
62.2	1.30212	33.61	10.836	67.2	1.33254	36.15	11.089
62.3	1.30273	33.67	10.841	67.3	1.33315	36.20	11.094
62.4	1.30334	33.72	10.846	67.4	1.33377	36.25	11.099
62.5	1.30393	33.77	10.851	67.5	1.33438	36.30	11.104
62.6	1.30453	33.82	10.856	67.6	1.33500	36.35	11.110
62.7	1.30513	33.87	10.861	67.7	1.33562	36.40	11.115
62.8	1.30573	33.92	10.866	67.8	1.33625	36.45	11.120
62.9	1.30633	33.97	10.871	67.9	1.33686	36.50	11.125

TABLE 3 (*Continued*)

Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.	Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.
68.0	1.33748	36.55	11.130	73.0	1.36900	39.05	11.392
68.1	1.33810	36.61	11.135	73.1	1.36964	39.10	11.398
68.2	1.33872	36.66	11.140	73.2	1.37028	39.15	11.403
68.3	1.33935	36.71	11.146	73.3	1.37092	39.20	11.408
68.4	1.33997	36.76	11.151	73.4	1.37156	39.25	11.414
68.5	1.34059	36.81	11.156	73.5	1.37220	39.30	11.419
68.6	1.34121	36.86	11.161	73.6	1.37283	39.35	11.424
68.7	1.34183	36.91	11.166	73.7	1.37347	39.39	11.430
68.8	1.34245	36.96	11.172	73.8	1.37411	39.44	11.435
68.9	1.34309	37.01	11.177	73.9	1.37476	39.49	11.440
69.0	1.34371	37.06	11.182	74.0	1.37541	39.54	11.446
69.1	1.34433	37.11	11.187	74.1	1.37605	39.59	11.451
69.2	1.34495	37.16	11.192	74.2	1.37669	39.64	11.456
69.3	1.34558	37.21	11.198	74.3	1.37733	36.69	11.462
69.4	1.34621	37.26	11.203	74.4	1.37798	39.74	11.467
69.5	1.34684	37.31	11.208	74.5	1.37864	39.79	11.473
69.6	1.34746	37.36	11.213	74.6	1.37928	39.84	11.478
69.7	1.34809	37.41	11.218	74.7	1.37993	39.89	11.483
69.8	1.34871	37.46	11.224	74.8	1.38057	39.94	11.489
69.9	1.34934	37.51	11.229	74.9	1.38122	39.99	11.494
70.0	1.34997	37.56	11.234	75.0	1.38187	40.03	11.499
70.1	1.35060	37.61	11.239	75.1	1.38252	40.08	11.505
70.2	1.35123	37.66	11.245	75.2	1.38316	40.13	11.510
70.3	1.35186	37.71	11.250	75.3	1.38381	40.18	11.516
70.4	1.35248	37.76	11.255	75.4	1.38445	40.23	11.521
70.5	1.35311	37.81	11.260	75.5	1.38510	40.28	11.526
70.6	1.35375	37.86	11.265	75.6	1.38575	40.33	11.532
70.7	1.35438	37.91	11.271	75.7	1.38640	40.38	11.537
70.8	1.35501	37.96	11.276	75.8	1.38705	40.43	11.543
70.9	1.35564	38.01	11.281	75.9	1.38770	40.48	11.548
71.0	1.35627	38.06	11.286	76.0	1.38835	40.53	11.554
71.1	1.35691	38.11	11.292	76.1	1.38902	40.57	11.559
71.2	1.35754	38.16	11.297	76.2	1.38967	40.62	11.564
71.3	1.35817	38.21	11.302	76.3	1.39032	40.67	11.570
71.4	1.35881	38.26	11.308	76.4	1.39097	40.72	11.575
71.5	1.35944	38.30	11.313	76.5	1.39162	40.77	11.581
71.6	1.36008	38.35	11.318	76.6	1.39228	40.82	11.586
71.7	1.36072	38.40	11.323	76.7	1.39293	40.87	11.592
71.8	1.36135	38.45	11.329	76.8	1.39358	40.92	11.597
71.9	1.36198	38.50	11.334	76.9	1.39423	40.97	11.602
72.0	1.36261	38.55	11.339	77.0	1.39489	41.01	11.608
72.1	1.36324	38.60	11.345	77.1	1.39554	41.06	11.613
72.2	1.36389	38.65	11.350	77.2	1.39619	41.11	11.619
72.3	1.36452	38.70	11.355	77.3	1.39685	41.16	11.624
72.4	1.36516	38.75	11.360	77.4	1.39750	41.21	11.630
72.5	1.36579	38.80	11.366	77.5	1.39816	41.26	11.636
72.6	1.36643	38.85	11.371	77.6	1.39882	41.31	11.641
72.7	1.36707	38.90	11.376	77.7	1.39949	41.36	11.646
72.8	1.36771	38.95	11.382	77.8	1.40014	41.40	11.652
72.9	1.36836	39.00	11.387	77.9	1.40080	41.45	11.657

TABLE 3 (*Continued*)

Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.	Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.
78.0	1.40146	41.50	11.663	83.0	1.43486	43.91	11.940
78.1	1.40211	41.55	11.668	83.1	1.43553	43.96	11.946
78.2	1.40277	41.60	11.673	83.2	1.43621	44.00	11.952
78.3	1.40344	41.65	11.679	83.3	1.43688	44.05	11.957
78.4	1.40409	41.70	11.684	83.4	1.43756	44.10	11.963
78.5	1.40475	41.74	11.690	83.5	1.43824	44.15	11.969
78.6	1.40541	41.79	11.695	83.6	1.43894	44.19	11.974
78.7	1.40607	41.84	11.701	83.7	1.43961	44.24	11.980
78.8	1.40674	41.89	11.706	83.8	1.44029	44.29	11.986
78.9	1.40740	41.94	11.712	83.9	1.44097	44.34	11.991
79.0	1.40806	41.99	11.717	84.0	1.44166	44.38	11.997
79.1	1.40872	42.03	11.723	84.1	1.44234	44.43	12.003
79.2	1.40938	42.08	11.728	84.2	1.44302	44.48	12.008
79.3	1.41005	42.13	11.734	84.3	1.44370	44.53	12.014
79.4	1.41072	42.18	11.740	84.4	1.44439	44.57	12.020
79.5	1.41138	42.23	11.745	84.5	1.44507	44.62	12.025
79.6	1.41204	42.28	11.751	84.6	1.44575	44.67	12.031
79.7	1.41270	42.32	11.756	84.7	1.44644	44.72	12.037
79.8	1.41337	42.37	11.762	84.8	1.44712	44.76	12.042
79.9	1.41404	42.42	11.767	84.9	1.44780	44.81	12.048
80.0	1.41471	42.47	11.773	85.0	1.44849	44.86	12.054
80.1	1.41537	42.52	11.778	85.1	1.44917	44.91	12.060
80.2	1.41603	42.57	11.784	85.2	1.44986	44.95	12.065
80.3	1.41670	42.61	11.789	85.3	1.45054	45.00	12.071
80.4	1.41737	42.66	11.795	85.4	1.45123	45.05	12.077
80.5	1.41804	42.71	11.801	85.5	1.45192	45.09	12.082
80.6	1.41872	42.76	11.806	85.6	1.45260	45.14	12.088
80.7	1.41937	42.81	11.812	85.7	1.45329	45.19	12.094
80.8	1.42004	42.85	11.817	85.8	1.45398	45.24	12.099
80.9	1.42072	42.90	11.823	85.9	1.45466	45.28	12.105
81.0	1.42138	42.95	11.828	86.0	1.45535	45.33	12.111
81.1	1.42205	43.00	11.834	86.1	1.45604	45.38	12.117
81.2	1.42272	43.05	11.839	86.2	1.45673	45.42	12.122
81.3	1.42339	43.10	11.845	86.3	1.45742	45.47	12.128
81.4	1.42406	43.14	11.851	86.4	1.45811	45.52	12.134
81.5	1.42474	43.19	11.856	86.5	1.45880	45.57	12.140
81.6	1.42541	43.24	11.862	86.6	1.45949	45.61	12.145
81.7	1.42608	43.29	11.867	86.7	1.46018	45.66	12.151
81.8	1.42675	43.33	11.873	86.8	1.46087	45.71	12.157
81.9	1.42742	43.38	11.879	86.9	1.46156	45.75	12.163
82.0	1.42810	43.43	11.884	87.0	1.46225	45.80	12.168
82.1	1.42878	43.48	11.890	87.1	1.46295	45.85	12.174
82.2	1.42946	43.53	11.895	87.2	1.46364	45.89	12.180
82.3	1.43013	43.57	11.901	87.3	1.46433	45.94	12.186
82.4	1.43080	43.62	11.907	87.4	1.46503	45.99	12.191
82.5	1.43148	43.67	11.912	87.5	1.46572	46.03	12.197
82.6	1.43214	43.72	11.918	87.6	1.46642	46.08	12.203
82.7	1.43282	43.77	11.924	87.7	1.46711	46.13	12.209
82.8	1.43350	43.81	11.929	87.8	1.46780	46.17	12.215
82.9	1.43417	43.86	11.935	87.9	1.46850	46.22	12.220

TABLE 3 (*Concluded*)

Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.	Per Cent Sucrose by Weight, or Degree Brix	Apparent Specific Gravity, 20°/20° C. (in air)	Degrees Baumé, Modulus 145, 20° C.	Pounds per Gallon, in Air, 20° C.
88.0	1.46919	46.27	12.226	92.0	1.49731	48.12	12.460
88.1	1.46989	46.31	12.232	92.1	1.49802	48.17	12.466
88.2	1.47059	46.36	12.238	92.2	1.49873	48.21	12.472
88.3	1.47128	46.41	12.243	92.3	1.49944	48.26	12.478
88.4	1.47198	46.45	12.249	92.4	1.50015	48.30	12.484
88.5	1.47268	46.50	12.255	92.5	1.50086	48.35	12.490
88.6	1.47338	46.55	12.261	92.6	1.50158	48.40	12.496
88.7	1.47407	46.59	12.267	92.7	1.50229	48.44	12.502
88.8	1.47477	46.64	12.273	92.8	1.50300	48.49	12.507
88.9	1.47547	46.69	12.278	92.9	1.50371	48.53	12.513
89.0	1.47617	46.73	12.284	93.0	1.50442	48.58	12.519
89.1	1.47687	46.78	12.290	93.1	1.50514	48.62	12.525
89.2	1.47757	46.83	12.296	93.2	1.50585	48.67	12.531
89.3	1.47827	46.87	12.302	93.3	1.50657	48.72	12.537
89.4	1.47897	46.92	12.307	93.4	1.50728	48.76	12.543
89.5	1.47967	46.97	12.313	93.5	1.50800	48.81	12.549
89.6	1.48037	47.01	12.319	93.6	1.50871	48.85	12.555
89.7	1.48107	47.06	12.325	93.7	1.50943	48.90	12.561
89.8	1.48177	47.11	12.331	93.8	1.51014	48.94	12.567
89.9	1.48248	47.15	12.337	93.9	1.51086	48.99	12.573
90.0	1.48318	47.20	12.342	94.0	1.51157	49.03	12.579
90.1	1.48388	47.24	12.348	94.1	1.51229	49.08	12.585
90.2	1.48459	47.29	12.354	94.2	1.51301	49.12	12.591
90.3	1.48529	47.34	12.360	94.3	1.51373	49.17	12.597
90.4	1.48600	47.38	12.366	94.4	1.51445	49.22	12.603
90.5	1.48670	47.43	12.372	94.5	1.51517	49.26	12.609
90.6	1.48740	47.48	12.378	94.6	1.51589	49.31	12.615
90.7	1.48811	47.52	12.383	94.7	1.51660	49.35	12.621
90.8	1.48881	47.57	12.389	94.8	1.51732	49.40	12.627
90.9	1.48952	47.61	12.395	94.9	1.51804	49.44	12.633
91.0	1.49022	47.66	12.401	95.0	1.51876	49.49	12.639
91.1	1.49093	47.71	12.407				
91.2	1.49164	47.75	12.413				
91.3	1.49235	47.80	12.419				
91.4	1.49306	47.84	12.425				
91.5	1.49376	47.89	12.431				
91.6	1.49447	47.94	12.437				
91.7	1.49518	47.98	12.442				
91.8	1.49589	48.03	12.448				
91.9	1.49660	48.08	12.454				

TABLE 4*

Temperature Corrections to Readings of Baumé Hydrometers, Bureau of Standards Baumé Scale for Sugar Solutions (Standard at 20° C.)

[This table is based on the values of the thermal expansion of sugar solutions by Plato, assuming the instrument to be of Jena 16III glass. The table should be used with caution and only for approximate results when the temperature differs much from the standard or from the temperature of the surrounding air.]

Temperature (°C.)	Observed Degrees Baumé								
	0	5	10	15	20	25	30	35	40
	Subtract from Observed Degrees Baumé								
0	0.17	0.34	0.47	0.57	0.65	0.72	0.77	0.79	0.81
5	.21	.30	.39	.45	.51	.55	.59	.60	.61
10	.18	.23	.28	.32	.36	.38	.40	.41	.42
11	.18	.22	.26	.29	.32	.34	.36	.37	.38
12	.17	.20	.23	.26	.29	.31	.32	.33	.34
13	.15	.18	.20	.23	.25	.27	.28	.29	.29
14	.14	.16	.18	.20	.22	.23	.24	.25	.25
15	.11	.13	.15	.17	.18	.20	.20	.21	.21
16	.10	.11	.13	.14	.15	.15	.16	.17	.17
17	.07	.08	.10	.11	.11	.11	.13	.13	.13
18	.05	.06	.07	.07	.08	.08	.08	.08	.09
19	.03	.03	.03	.03	.04	.04	.04	.04	.05
	Add to Observed Degrees Baumé								
21	0.02	0.03	0.03	0.04	0.04	0.04	0.04	0.05	0.05
22	.06	.06	.07	.07	.08	.08	.08	.09	.09
23	.09	.09	.10	.11	.12	.13	.13	.13	.13
24	.12	.13	.14	.15	.16	.17	.17	.17	.17
25	.15	.17	.18	.19	.20	.21	.21	.21	.21
26	.19	.20	.22	.22	.24	.26	.26	.26	.26
27	.23	.23	.25	.27	.29	.30	.30	.30	.30
28	.26	.27	.29	.31	.33	.34	.35	.35	.34
29	.31	.31	.34	.35	.37	.38	.39	.39	.38
30	.35	.35	.38	.39	.42	.43	.44	.43	.43
35	.56	.57	.60	.63	.65	.66	.66	.66	.65
40	.81	.82	.85	.87	.89	.90	.90	.89	.88
45	1.09	1.09	1.12	1.14	1.15	1.15	1.14	1.13	1.11
50	1.40	1.39	1.42	1.42	1.43	1.41	1.40	1.37	1.33
55	1.74	1.72	1.73	1.73	1.71	1.69	1.66	1.62	1.57
60	2.10	2.08	2.06	2.05	2.00	1.97	1.92	1.87	1.82
65	2.5	2.5	2.4	2.4	2.3	2.2	2.2	2.1	2.1
70	2.9	2.8	2.8	2.8	2.6	2.6	2.5	2.4	2.3
75	3.5	3.3	3.2	3.2	3.1	3.0	2.9	2.7	2.6
80	4.0	3.9	3.8	3.7	3.5	3.4	3.3	3.1	2.8

* Taken from *Bur. Standards Cir.* 295, 1926. See also text, p. 82.

TABLE 5*

Weights per United States Gallon of Sucrose Solutions at Different Temperatures

Per Cent Sucrose by Weight (Brix)	Weights per Gallon in Air at t° C.				
	t = 10° C. Pounds	t = 15° C. Pounds	t = 20° C. Pounds	t = 25° C. Pounds	t = 30° C. Pounds
0	8.334	8.329	8.322	8.312	8.301
5	8.500	8.494	8.485	8.475	8.463
10	8.672	8.664	8.655	8.644	8.631
15	8.849	8.841	8.830	8.818	8.805
20	9.034	9.023	9.012	8.999	8.985
25	9.225	9.213	9.201	9.187	9.171
30	9.423	9.410	9.396	9.381	9.365
35	9.628	9.614	9.599	9.583	9.566
40	9.840	9.825	9.809	9.792	9.774
45	10.060	10.044	10.027	10.009	9.990
50	10.288	10.271	10.252	10.234	10.214
55	10.523	10.505	10.486	10.466	10.446
60	10.767	10.747	10.727	10.707	10.685
65	11.018	10.997	10.977	10.955	10.933
70	11.277	11.256	11.234	11.212	11.189
75	*11.544*	11.522	11.499	*11.477*	*11.453*
80	*11.818*	11.796	11.773	*11.749*	*11.725*
85	*12.101*	12.078	12.054	*12.030*	*12.006*
90	*12.391*	12.367	12.342	*12.318*	*12.293*
95	*12.688*	12.664	12.639	*12.613*	*12.587*

*Taken from *Bur. Standards Cir.* 375, 1929. See also text, p. 66. The figures in italics were derived by extrapolation.

In the calculation of this table the density of air (at 20° C., and barometer reading 760 mm. of mercury) was taken as 0.0012. The effect of differences of barometric pressure on the weights of a gallon of a 70 per cent solution of sucrose in air is shown below.

Weight in Air of 1 Gallon of a 70 Per Cent Solution of Sucrose at 20° C. at Different Barometric Pressures

Pressure mm. Hg	Density Air	Weight per Gallon in Air, Pounds
780	0.00124	11.234
760	.00120	11.234
740	.00117	11.234
720	.00114	11.235
700	.00111	11.235
680	.00108	11.235
660	.00105	11.235

TABLE 6*

International Scale (1936) of Refractive Indices of Sucrose Solutions

Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}
0.0	1.33299	1.33219	4.0	1.33880	1.33795	8.0	1.34477	1.34387
0.1	1.33313	1.33233	4.1	1.33895	1.33810	8.1	1.34492	1.34402
0.2	1.33328	1.33248	4.2	1.33909	1.33824	8.2	1.34507	1.34417
0.3	1.33342	1.33262	4.3	1.33924	1.33839	8.3	1.34523	1.34432
0.4	1.33357	1.33276	4.4	1.33939	1.33853	8.4	1.34538	1.34447
0.5	1.33371	1.33291	4.5	1.33953	1.33868	8.5	1.34553	1.34463
0.6	1.33385	1.33305	4.6	1.33968	1.33883	8.6	1.34568	1.34478
0.7	1.33400	1.33319	4.7	1.33983	1.33897	8.7	1.34583	1.34493
0.8	1.33414	1.33333	4.8	1.33998	1.33912	8.8	1.34599	1.34508
0.9	1.33429	1.33348	4.9	1.34012	1.33926	8.9	1.34614	1.34523
1.0	1.33443	1.33362	5.0	1.34027	1.33941	9.0	1.34629	1.34538
1.1	1.33457	1.33376	5.1	1.34042	1.33956	9.1	1.34644	1.34553
1.2	1.33472	1.33391	5.2	1.34057	1.33971	9.2	1.34660	1.34569
1.3	1.33487	1.33405	5.3	1.34072	1.33985	9.3	1.34675	1.34584
1.4	1.33501	1.33420	5.4	1.34087	1.34000	9.4	1.34691	1.34599
1.5	1.33515	1.33434	5.5	1.34101	1.34015	9.5	1.34706	1.34615
1.6	1.33530	1.33448	5.6	1.34116	1.34030	9.6	1.34721	1.34630
1.7	1.33545	1.33463	5.7	1.34131	1.34045	9.7	1.34737	1.34645
1.8	1.33559	1.33477	5.8	1.34146	1.34059	9.8	1.34752	1.34660
1.9	1.33573	1.33492	5.9	1.34161	1.34074	9.9	1.34768	1.34676
2.0	1.33588	1.33506	6.0	1.34176	1.34089	10.0	1.34783	1.34691
2.1	1.33603	1.33520	6.1	1.34191	1.34104	10.1	1.34798	1.34706
2.2	1.33617	1.33535	6.2	1.34206	1.34119	10.2	1.34814	1.34722
2.3	1.33631	1.33549	6.3	1.34221	1.34134	10.3	1.34829	1.34737
2.4	1.33646	1.33563	6.4	1.34236	1.34149	10.4	1.34845	1.34752
2.5	1.33661	1.33577	6.5	1.34251	1.34163	10.5	1.34860	1.34767
2.6	1.33675	1.33592	6.6	1.34266	1.34178	10.6	1.34875	1.34783
2.7	1.33689	1.33606	6.7	1.34281	1.34193	10.7	1.34891	1.34798
2.8	1.33704	1.33620	6.8	1.34296	1.34208	10.8	1.34906	1.34813
2.9	1.33719	1.33635	6.9	1.34311	1.34223	10.9	1.34922	1.34829
3.0	1.33733	1.33649	7.0	1.34326	1.34238	11.0	1.34937	1.34844
3.1	1.33748	1.33664	7.1	1.34341	1.34253	11.1	1.34953	1.34859
3.2	1.33762	1.33678	7.2	1.34356	1.34268	11.2	1.34968	1.34875
3.3	1.33777	1.33693	7.3	1.34371	1.34283	11.3	1.34984	1.34891
3.4	1.33792	1.33707	7.4	1.34386	1.34298	11.4	1.34999	1.34906
3.5	1.33807	1.33722	7.5	1.34401	1.34313	11.5	1.35015	1.34921
3.6	1.33821	1.33737	7.6	1.34417	1.34327	11.6	1.35031	1.34937
3.7	1.33836	1.33751	7.7	1.34432	1.34342	11.7	1.35046	1.34953
3.8	1.33851	1.33766	7.8	1.34447	1.34357	11.8	1.35062	1.34968
3.9	1.33865	1.33780	7.9	1.34462	1.34372	11.9	1.35077	1.34983

* See text, p. 102. Taken from *Proceedings* of the Ninth Session of the International Commission for Uniform Methods of Sugar Analysis, London, 1936 (*Intern. Sugar J.*, **39**, 23s). The refractive indices for tenths of per cent have been interpolated linearly and checked against data of the National Bureau of Standards.

TABLE 6 (*Continued*)

Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}
12.0	1.35093	1.34999	17.0	1.35890	1.35791	22.0	1.36719	1.36615
12.1	1.35109	1.35015	17.1	1.35906	1.35807	22.1	1.36736	1.36632
12.2	1.35124	1.35030	17.2	1.35923	1.35823	22.2	1.36753	1.36648
12.3	1.35140	1.35046	17.3	1.35939	1.35840	22.3	1.36770	1.36665
12.4	1.35156	1.35061	17.4	1.35955	1.35856	22.4	1.36787	1.36682
12.5	1.35171	1.35077	17.5	1.35971	1.35872	22.5	1.36803	1.36699
12.6	1.35187	1.35093	17.6	1.35988	1.35888	22.6	1.36820	1.36715
12.7	1.35203	1.35108	17.7	1.36004	1.35904	22.7	1.36837	1.36732
12.8	1.35219	1.35124	17.8	1.36020	1.35921	22.8	1.36854	1.36749
12.9	1.35234	1.35139	17.9	1.36037	1.35937	22.9	1.36871	1.36765
13.0	1.35250	1.35155	18.0	1.36053	1.35953	23.0	1.36888	1.36782
13.1	1.35266	1.35171	18.1	1.36069	1.35969	23.1	1.36905	1.36799
13.2	1.35282	1.35186	18.2	1.36086	1.35986	23.2	1.36922	1.36816
13.3	1.35297	1.35202	18.3	1.36103	1.36002	23.3	1.36939	1.36833
13.4	1.35313	1.35218	18.4	1.36119	1.36019	23.4	1.36956	1.36850
13.5	1.35329	1.35233	18.5	1.36135	1.36035	23.5	1.36973	1.36867
13.6	1.35345	1.35249	18.6	1.36152	1.36051	23.6	1.36991	1.36884
13.7	1.35361	1.35265	18.7	1.36169	1.36068	23.7	1.37008	1.36901
13.8	1.35376	1.35281	18.8	1.36185	1.36084	23.8	1.37025	1.36918
13.9	1.35392	1.35296	18.9	1.36201	1.36101	23.9	1.37042	1.36935
14.0	1.35408	1.35312	19.0	1.36218	1.36117	24.0	1.37059	1.36952
14.1	1.35424	1.35328	19.1	1.36235	1.36133	24.1	1.3708	1.3697
14.2	1.35440	1.35344	19.2	1.36251	1.36150	24.2	1.3709	1.3699
14.3	1.35456	1.35359	19.3	1.36268	1.36167	24.3	1.3711	1.3700
14.4	1.35472	1.35375	19.4	1.36284	1.36183	24.4	1.3713	1.3702
14.5	1.35487	1.35391	19.5	1.36301	1.36199	24.5	1.3715	1.3704
14.6	1.35503	1.35407	19.6	1.36318	1.36216	24.6	1.3716	1.3705
14.7	1.35519	1.35423	19.7	1.36334	1.36233	24.7	1.3718	1.3707
14.8	1.35535	1.35438	19.8	1.36351	1.36249	24.8	1.3720	1.3709
14.9	1.35551	1.35454	19.9	1.36367	1.36265	24.9	1.3721	1.3710
15.0	1.35567	1.35470	20.0	1.36384	1.36282	25.0	1.3723	1.3712
15.1	1.35583	1.35486	20.1	1.36401	1.36299	25.1	1.3725	1.3714
15.2	1.35599	1.35502	20.2	1.36417	1.36315	25.2	1.3726	1.3715
15.3	1.35615	1.35518	20.3	1.36434	1.36332	25.3	1.3728	1.3717
15.4	1.35631	1.35534	20.4	1.36451	1.36348	25.4	1.3730	1.3719
15.5	1.35647	1.35550	20.5	1.36467	1.36365	25.5	1.3731	1.3721
15.6	1.35664	1.35566	20.6	1.36484	1.36382	25.6	1.3733	1.3722
15.7	1.35680	1.35582	20.7	1.36501	1.36398	25.7	1.3735	1.3724
15.8	1.35696	1.35598	20.8	1.36518	1.36415	25.8	1.3737	1.3726
15.9	1.35712	1.35614	20.9	1.36534	1.36431	25.9	1.3738	1.3727
16.0	1.35728	1.35630	21.0	1.36551	1.36448	26.0	1.3740	1.3729
16.1	1.35744	1.35646	21.1	1.36568	1.36465	26.1	1.3742	1.3731
16.2	1.35760	1.35662	21.2	1.36585	1.36481	26.2	1.3744	1.3733
16.3	1.35777	1.35678	21.3	1.36601	1.36498	26.3	1.3745	1.3734
16.4	1.35793	1.35694	21.4	1.36618	1.36515	26.4	1.3747	1.3736
16.5	1.35809	1.35711	21.5	1.36635	1.36531	26.5	1.3749	1.3738
16.6	1.35825	1.35727	21.6	1.36652	1.36548	26.6	1.3751	1.3740
16.7	1.35841	1.35743	21.7	1.36669	1.36565	26.7	1.3753	1.3742
16.8	1.35858	1.35759	21.8	1.36685	1.36582	26.8	1.3754	1.3743
16.9	1.35874	1.35775	21.9	1.36702	1.36598	26.9	1.3756	1.3745

TABLE 6 (*Continued*)

Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}
27.0	1.3758	1.3747	32.0	1.3847	1.3835	37.0	1.3939	1.3927
27.1	1.3760	1.3749	32.1	1.3849	1.3837	37.1	1.3941	1.3929
27.2	1.3761	1.3750	32.2	1.3851	1.3839	37.2	1.3943	1.3931
27.3	1.3763	1.3752	32.3	1.3852	1.3840	37.3	1.3945	1.3933
27.4	1.3765	1.3754	32.4	1.3854	1.3842	37.4	1.3947	1.3935
27.5	1.3767	1.3756	32.5	1.3856	1.3844	37.5	1.3949	1.3937
27.6	1.3768	1.3757	32.6	1.3858	1.3846	37.6	1.3950	1.3938
27.7	1.3770	1.3759	32.7	1.3860	1.3848	37.7	1.3952	1.3940
27.8	1.3772	1.3761	32.8	1.3861	1.3849	37.8	1.3954	1.3942
27.9	1.3773	1.3762	32.9	1.3863	1.3851	37.9	1.3956	1.3944
28.0	1.3775	1.3764	33.0	1.3865	1.3853	38.0	1.3958	1.3946
28.1	1.3777	1.3766	33.1	1.3867	1.3855	38.1	1.3960	1.3948
28.2	1.3779	1.3768	33.2	1.3869	1.3857	38.2	1.3962	1.3950
28.3	1.3780	1.3769	33.3	1.3870	1.3858	38.3	1.3964	1.3952
28.4	1.3782	1.3771	33.4	1.3872	1.3860	38.4	1.3966	1.3954
28.5	1.3784	1.3773	33.5	1.3874	1.3862	38.5	1.3968	1.3956
28.6	1.3786	1.3775	33.6	1.3876	1.3864	38.6	1.3970	1.3958
28.7	1.3788	1.3777	33.7	1.3878	1.3866	38.7	1.3972	1.3960
28.8	1.3789	1.3778	33.8	1.3879	1.3867	38.8	1.3974	1.3962
28.9	1.3791	1.3780	33.9	1.3881	1.3869	38.9	1.3976	1.3964
29.0	1.3793	1.3782	34.0	1.3883	1.3871	39.0	1.3978	1.3966
29.1	1.3795	1.3784	34.1	1.3885	1.3873	39.1	1.3980	1.3968
29.2	1.3797	1.3786	34.2	1.3887	1.3875	39.2	1.3982	1.3970
29.3	1.3798	1.3787	34.3	1.3889	1.3877	39.3	1.3984	1.3972
29.4	1.3800	1.3789	34.4	1.3891	1.3879	39.4	1.3986	1.3974
29.5	1.3802	1.3791	34.5	1.3893	1.3881	39.5	1.3987	1.3975
29.6	1.3804	1.3793	34.6	1.3894	1.3882	39.6	1.3989	1.3977
29.7	1.3806	1.3795	34.7	1.3896	1.3884	39.7	1.3991	1.3979
29.8	1.3807	1.3796	34.8	1.3898	1.3886	39.8	1.3993	1.3981
29.9	1.3809	1.3798	34.9	1.3900	1.3888	39.9	1.3995	1.3983
30.0	1.3811	1.3800	35.0	1.3902	1.3890	40.0	1.3997	1.3985
30.1	1.3813	1.3802	35.1	1.3904	1.3892	40.1	1.3999	1.3987
30.2	1.3815	1.3804	35.2	1.3906	1.3894	40.2	1.4001	1.3989
30.3	1.3816	1.3805	35.3	1.3907	1.3895	40.3	1.4003	1.3990
30.4	1.3818	1.3807	35.4	1.3909	1.3897	40.4	1.4005	1.3992
30.5	1.3820	1.3809	35.5	1.3911	1.3899	40.5	1.4007	1.3994
30.6	1.3822	1.3811	35.6	1.3913	1.3901	40.6	1.4008	1.3996
30.7	1.3824	1.3813	35.7	1.3915	1.3903	40.7	1.4010	1.3998
30.8	1.3825	1.3814	35.8	1.3916	1.3904	40.8	1.4012	1.3999
30.9	1.3827	1.3816	35.9	1.3918	1.3906	40.9	1.4014	1.4001
31.0	1.3829	1.3818	36.0	1.3920	1.3908	41.0	1.4016	1.4003
31.1	1.3831	1.3820	36.1	1.3922	1.3910	41.1	1.4018	1.4005
31.2	1.3833	1.3821	36.2	1.3924	1.3912	41.2	1.4020	1.4007
31.3	1.3834	1.3823	36.3	1.3926	1.3914	41.3	1.4022	1.4009
31.4	1.3836	1.3825	36.4	1.3928	1.3916	41.4	1.4024	1.4011
31.5	1.3838	1.3827	36.5	1.3929	1.3917	41.5	1.4026	1.4013
31.6	1.3840	1.3828	36.6	1.3931	1.3919	41.6	1.4028	1.4015
31.7	1.3842	1.3830	36.7	1.3933	1.3921	41.7	1.4030	1.4017
31.8	1.3843	1.3832	36.8	1.3935	1.3923	41.8	1.4032	1.4019
31.9	1.3845	1.3833	36.9	1.3937	1.3925	41.9	1.4034	1.4021

TABLE 6 (*Continued*)

Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}
42.0	1.4036	1.4023	47.0	1.4137	1.4124	52.0	1.4242	1.4228
42.1	1.4038	1.4025	47.1	1.4139	1.4126	52.1	1.4244	1.4230
42.2	1.4040	1.4027	47.2	1.4141	1.4128	52.2	1.4246	1.4232
42.3	1.4042	1.4029	47.3	1.4143	1.4130	52.3	1.4249	1.4235
42.4	1.4044	1.4031	47.4	1.4145	1.4132	52.4	1.4251	1.4237
42.5	1.4046	1.4033	47.5	1.4147	1.4135	52.5	1.4253	1.4239
42.6	1.4048	1.4035	47.6	1.4150	1.4137	52.6	1.4255	1.4241
42.7	1.4050	1.4037	47.7	1.4152	1.4139	52.7	1.4257	1.4243
42.8	1.4052	1.4039	47.8	1.4154	1.4141	52.8	1.4260	1.4246
42.9	1.4054	1.4041	47.9	1.4156	1.4143	52.9	1.4262	1.4248
43.0	1.4056	1.4043	48.0	1.4158	1.4145	53.0	1.4264	1.4250
43.1	1.4058	1.4045	48.1	1.4160	1.4147	53.1	1.4266	1.4252
43.2	1.4060	1.4047	48.2	1.4162	1.4149	53.2	1.4268	1.4254
43.3	1.4062	1.4049	48.3	1.4164	1.4151	53.3	1.4270	1.4256
43.4	1.4064	1.4051	48.4	1.4166	1.4153	53.4	1.4272	1.4258
43.5	1.4066	1.4053	48.5	1.4169	1.4155	53.5	1.4275	1.4261
43.6	1.4068	1.4055	48.6	1.4171	1.4158	53.6	1.4277	1.4263
43.7	1.4070	1.4057	48.7	1.4173	1.4160	53.7	1.4279	1.4265
43.8	1.4072	1.4059	48.8	1.4175	1.4162	53.8	1.4281	1.4267
43.9	1.4074	1.4061	48.9	1.4177	1.4164	53.9	1.4283	1.4269
44.0	1.4076	1.4063	49.0	1.4179	1.4166	54.0	1.4285	1.4271
44.1	1.4078	1.4065	49.1	1.4181	1.4168	54.1	1.4287	1.4273
44.2	1.4080	1.4067	49.2	1.4183	1.4170	54.2	1.4289	1.4275
44.3	1.4082	1.4069	49.3	1.4185	1.4172	54.3	1.4292	1.4278
44.4	1.4084	1.4071	49.4	1.4187	1.4174	54.4	1.4294	1.4280
44.5	1.4086	1.4073	49.5	1.4189	1.4177	54.5	1.4296	1.4282
44.6	1.4088	1.4075	49.6	1.4192	1.4179	54.6	1.4298	1.4284
44.7	1.4090	1.4077	49.7	1.4194	1.4181	54.7	1.4300	1.4286
44.8	1.4092	1.4079	49.8	1.4196	1.4183	54.8	1.4303	1.4289
44.9	1.4094	1.4081	49.9	1.4198	1.4185	54.9	1.4305	1.4291
45.0	1.4096	1.4083	50.0	1.4200	1.4187	55.0	1.4307	1.4293
45.1	1.4098	1.4085	50.1	1.4202	1.4189	55.1	1.4309	1.4295
45.2	1.4100	1.4087	50.2	1.4204	1.4191	55.2	1.4311	1.4297
45.3	1.4102	1.4089	50.3	1.4206	1.4193	55.3	1.4313	1.4300
45.4	1.4104	1.4091	50.4	1.4208	1.4195	55.4	1.4316	1.4302
45.5	1.4107	1.4093	50.5	1.4211	1.4197	55.5	1.4318	1.4304
45.6	1.4109	1.4096	50.6	1.4213	1.4199	55.6	1.4320	1.4306
45.7	1.4111	1.4098	50.7	1.4215	1.4201	55.7	1.4322	1.4308
45.8	1.4113	1.4100	50.8	1.4217	1.4203	55.8	1.4325	1.4311
45.9	1.4115	1.4102	50.9	1.4219	1.4205	55.9	1.4327	1.4313
46.0	1.4117	1.4104	51.0	1.4221	1.4207	56.0	1.4329	1.4315
46.1	1.4119	1.4106	51.1	1.4223	1.4209	56.1	1.4331	1.4317
46.2	1.4121	1.4108	51.2	1.4225	1.4211	56.2	1.4333	1.4319
46.3	1.4123	1.4110	51.3	1.4227	1.4213	56.3	1.4336	1.4322
46.4	1.4125	1.4112	51.4	1.4229	1.4215	56.4	1.4338	1.4324
46.5	1.4127	1.4114	51.5	1.4231	1.4217	56.5	1.4340	1.4326
46.6	1.4129	1.4116	51.6	1.4234	1.4220	56.6	1.4342	1.4328
46.7	1.4131	1.4118	51.7	1.4236	1.4222	56.7	1.4344	1.4330
46.8	1.4133	1.4120	51.8	1.4238	1.4224	56.8	1.4347	1.4333
46.9	1.4135	1.4122	51.9	1.4240	1.4226	56.9	1.4349	1.4335

TABLE 6 (*Continued*)

Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}
57.0	1.4351	1.4337	62.0	1.4464	1.4449	67.0	1.4579	1.4564
57.1	1.4353	1.4339	62.1	1.4466	1.4451	67.1	1.4581	1.4566
57.2	1.4355	1.4341	62.2	1.4468	1.4453	67.2	1.4584	1.4569
57.3	1.4358	1.4344	62.3	1.4471	1.4455	67.3	1.4586	1.4571
57.4	1.4360	1.4346	62.4	1.4473	1.4458	67.4	1.4589	1.4574
57.5	1.4362	1.4348	62.5	1.4475	1.4460	67.5	1.4591	1.4576
57.6	1.4364	1.4350	62.6	1.4477	1.4462	67.6	1.4593	1.4578
57.7	1.4366	1.4352	62.7	1.4479	1.4464	67.7	1.4596	1.4581
57.8	1.4369	1.4355	62.8	1.4482	1.4467	67.8	1.4598	1.4583
57.9	1.4371	1.4357	62.9	1.4484	1.4469	67.9	1.4601	1.4586
58.0	1.4373	1.4359	63.0	1.4486	1.4471	68.0	1.4603	1.4588
58.1	1.4375	1.4361	63.1	1.4488	1.4473	68.1	1.4605	1.4590
58.2	1.4378	1.4364	63.2	1.4491	1.4476	68.2	1.4608	1.4593
58.3	1.4380	1.4366	63.3	1.4493	1.4478	68.3	1.4610	1.4595
58.4	1.4382	1.4368	63.4	1.4495	1.4480	68.4	1.4613	1.4598
58.5	1.4385	1.4371	63.5	1.4497	1.4483	68.5	1.4615	1.4600
58.6	1.4387	1.4373	63.6	1.4500	1.4485	68.6	1.4617	1.4602
58.7	1.4389	1.4375	63.7	1.4502	1.4487	68.7	1.4620	1.4605
58.8	1.4391	1.4377	63.8	1.4504	1.4489	68.8	1.4622	1.4607
58.9	1.4394	1.4380	63.9	1.4507	1.4492	68.9	1.4625	1.4610
59.0	1.4396	1.4382	64.0	1.4509	1.4494	69.0	1.4627	1.4612
59.1	1.4398	1.4384	64.1	1.4511	1.4496	69.1	1.4629	1.4614
59.2	1.4400	1.4386	64.2	1.4514	1.4499	69.2	1.4632	1.4617
59.3	1.4403	1.4388	64.3	1.4516	1.4501	69.3	1.4634	1.4619
59.4	1.4405	1.4390	64.4	1.4518	1.4503	69.4	1.4637	1.4621
59.5	1.4407	1.4393	64.5	1.4521	1.4505	69.5	1.4639	1.4623
59.6	1.4409	1.4395	64.6	1.4523	1.4508	69.6	1.4641	1.4626
59.7	1.4411	1.4397	64.7	1.4525	1.4510	69.7	1.4644	1.4628
59.8	1.4414	1.4399	64.8	1.4527	1.4512	69.8	1.4646	1.4630
59.9	1.4416	1.4401	64.9	1.4530	1.4515	69.9	1.4649	1.4633
60.0	1.4418	1.4403	65.0	1.4532	1.4517	70.0	1.4651	1.4635
60.1	1.4420	1.4405	65.1	1.4534	1.4519	70.1	1.4653	1.4637
60.2	1.4423	1.4408	65.2	1.4537	1.4522	70.2	1.4656	1.4640
60.3	1.4425	1.4410	65.3	1.4539	1.4524	70.3	1.4658	1.4643
60.4	1.4427	1.4412	65.4	1.4541	1.4526	70.4	1.4661	1.4645
60.5	1.4429	1.4415	65.5	1.4544	1.4529	70.5	1.4663	1.4647
60.6	1.4432	1.4417	65.6	1.4546	1.4531	70.6	1.4666	1.4650
60.7	1.4434	1.4419	65.7	1.4548	1.4533	70.7	1.4668	1.4653
60.8	1.4436	1.4421	65.8	1.4550	1.4535	70.8	1.4671	1.4655
60.9	1.4439	1.4424	65.9	1.4553	1.4538	70.9	1.4673	1.4657
61.0	1.4441	1.4426	66.0	1.4555	1.4540	71.0	1.4676	1.4660
61.1	1.4443	1.4428	66.1	1.4557	1.4542	71.1	1.4678	1.4662
61.2	1.4446	1.4431	66.2	1.4560	1.4545	71.2	1.4681	1.4665
61.3	1.4448	1.4433	66.3	1.4562	1.4547	71.3	1.4683	1.4667
61.4	1.4450	1.4435	66.4	1.4565	1.4550	71.4	1.4685	1.4670
61.5	1.4453	1.4437	66.5	1.4567	1.4552	71.5	1.4688	1.4672
61.6	1.4455	1.4440	66.6	1.4569	1.4554	71.6	1.4690	1.4674
61.7	1.4457	1.4442	66.7	1.4572	1.4557	71.7	1.4693	1.4677
61.8	1.4459	1.4444	66.8	1.4574	1.4559	71.8	1.4695	1.4679
61.9	1.4462	1.4447	66.9	1.4577	1.4562	71.9	1.4698	1.4682

TABLE 6 (*Concluded*)

Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}	Per Cent Sucrose by Weight	n_D^{20}	n_D^{28}
72.0	1.4700	1.4684	77.0	1.4825	1.4809	82.0	1.4954	1.4937
72.1	1.4703	1.4687	77.1	1.4827	1.4811	82.1	1.4956	1.4940
72.2	1.4705	1.4689	77.2	1.4830	1.4814	82.2	1.4959	1.4942
72.3	1.4708	1.4691	77.3	1.4832	1.4817	82.3	1.4962	1.4945
72.4	1.4710	1.4694	77.4	1.4835	1.4819	82.4	1.4964	1.4947
72.5	1.4713	1.4697	77.5	1.4838	1.4821	82.5	1.4967	1.4950
72.6	1.4715	1.4699	77.6	1.4840	1.4824	82.6	1.4970	1.4953
72.7	1.4717	1.4701	77.7	1.4843	1.4827	82.7	1.4972	1.4955
72.8	1.4720	1.4704	77.8	1.4845	1.4829	82.8	1.4975	1.4958
72.9	1.4722	1.4707	77.9	1.4848	1.4831	82.9	1.4978	1.4960
73.0	1.4725	1.4709	78.0	1.4850	1.4834	83.0	1.4980	1.4963
73.1	1.4727	1.4711	78.1	1.4853	1.4837	83.1	1.4983	1.4966
73.2	1.4730	1.4714	78.2	1.4855	1.4839	83.2	1.4985	1.4968
73.3	1.4732	1.4716	78.3	1.4858	1.4842	83.3	1.4988	1.4971
73.4	1.4735	1.4719	78.4	1.4860	1.4844	83.4	1.4991	1.4974
73.5	1.4737	1.4721	78.5	1.4863	1.4847	83.5	1.4993	1.4977
73.6	1.4740	1.4723	78.6	1.4865	1.4850	83.6	1.4996	1.4979
73.7	1.4742	1.4726	78.7	1.4868	1.4852	83.7	1.4999	1.4982
73.8	1.4744	1.4728	78.8	1.4871	1.4855	83.8	1.5001	1.4985
73.9	1.4747	1.4731	78.9	1.4873	1.4857	83.9	1.5004	1.4987
74.0	1.4749	1.4733	79.0	1.4876	1.4860	84.0	1.5007	1.4990
74.1	1.4752	1.4735	79.1	1.4878	1.4862	84.1	1.5009	1.4993
74.2	1.4754	1.4738	79.2	1.4881	1.4865	84.2	1.5012	1.4995
74.3	1.4757	1.4741	79.3	1.4883	1.4867	84.3	1.5015	1.4998
74.4	1.4759	1.4743	79.4	1.4886	1.4870	84.4	1.5017	1.5000
74.5	1.4762	1.4745	79.5	1.4888	1.4872	84.5	1.5020	1.5003
74.6	1.4764	1.4748	79.6	1.4891	1.4874	84.6	1.5022	1.5006
74.7	1.4767	1.4751	79.7	1.4893	1.4877	84.7	1.5025	1.5008
74.8	1.4769	1.4753	79.8	1.4896	1.4879	84.8	1.5028	1.5011
74.9	1.4772	1.4755	79.9	1.4898	1.4882	84.9	1.5030	1.5013
75.0	1.4774	1.4758	80.0	1.4901	1.4884	85.0	1.5033	1.5016
75.1	1.4777	1.4761	80.1	1.4904	1.4887			
75.2	1.4779	1.4763	80.2	1.4906	1.4889			
75.3	1.4782	1.4765	80.3	1.4909	1.4892			
75.4	1.4784	1.4768	80.4	1.4912	1.4894			
75.5	1.4787	1.4771	80.5	1.4914	1.4897			
75.6	1.4789	1.4773	80.6	1.4917	1.4900			
75.7	1.4792	1.4775	80.7	1.4919	1.4902			
75.8	1.4794	1.4778	80.8	1.4922	1.4905			
75.9	1.4797	1.4781	80.9	1.4925	1.4907			
76.0	1.4799	1.4783	81.0	1.4927	1.4910			
76.1	1.4802	1.4786	81.1	1.4930	1.4913			
76.2	1.4804	1.4788	81.2	1.4933	1.4915			
76.3	1.4807	1.4791	81.3	1.4935	1.4918			
76.4	1.4810	1.4793	81.4	1.4938	1.4921			
76.5	1.4812	1.4796	81.5	1.4941	1.4923			
76.6	1.4815	1.4799	81.6	1.4943	1.4926			
76.7	1.4817	1.4801	81.7	1.4946	1.4929			
76.8	1.4820	1.4804	81.8	1 4949	1.4932			
76.9	1.4822	1.4806	81.9	1.4951	1.4934			

TABLE 7*

INTERNATIONAL TEMPERATURE CORRECTION TABLE (1936) FOR THE 20° MODEL OF REFRACTOMETER, ABOVE AND BELOW 20° C.

Temp. °C.	Per Cent Sucrose														
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70
	Subtract from the Per Cent Sucrose														
10	0.50	0.54	0.58	0.61	0.64	0.66	0.68	0.70	0.72	0.73	0.74	0.75	0.76	0.78	0.79
11	0.46	0.49	0.53	0.55	0.58	0.60	0.62	0.64	0.65	0.66	0.67	0.68	0.69	0.70	0.71
12	0.42	0.45	0.48	0.50	0.52	0.54	0.56	0.57	0.58	0.59	0.60	0.61	0.61	0.63	0.63
13	0.37	0.40	0.42	0.44	0.46	0.48	0.49	0.50	0.51	0.52	0.53	0.54	0.54	0.55	0.55
14	0.33	0.35	0.37	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.45	0.46	0.46	0.47	0.48
15	0.27	0.29	0.31	0.33	0.34	0.34	0.35	0.36	0.37	0.37	0.38	0.39	0.39	0.40	0.40
16	0.22	0.24	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.30	0.30	0.31	0.31	0.32	0.32
17	0.17	0.18	0.19	0.20	0.21	0.21	0.21	0.22	0.22	0.23	0.23	0.23	0.23	0.24	0.24
18	0.12	0.13	0.13	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16
19	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
	Add to the Per Cent Sucrose														
21	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
22	0.13	0.13	0.14	0.14	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.16	0.16
23	0.19	0.20	0.21	0.22	0.22	0.23	0.23	0.23	0.23	0.24	0.24	0.24	0.24	0.24	0.24
24	0.26	0.27	0.28	0.29	0.30	0.30	0.31	0.31	0.31	0.31	0.31	0.32	0.32	0.32	0.32
25	0.33	0.35	0.36	0.37	0.38	0.38	0.39	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
26	0.40	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
27	0.48	0.50	0.52	0.53	0.54	0.55	0.55	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
28	0.56	0.57	0.60	0.61	0.62	0.63	0.63	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64
29	0.64	0.66	0.68	0.69	0.71	0.72	0.72	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
30	0.72	0.74	0.77	0.78	0.79	0.80	0.80	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81

* See text, p. 102. Taken from *Proceedings* of the Ninth Session of the International Commission for Uniform Methods of Sugar Analysis, London, 1936 (*Intern. Sugar J.*, **39**, 24a).

TABLE 8*

International Temperature Correction Table (1936) for the tropical model of refractometer, above and below 28° C.

Temp. °C.	Per Cent Sucrose														
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70
	Subtract from the Per Cent Sucrose														
20	0.57	0.59	0.60	0.61	0.62	0.63	0.63	0.64	0.64	0.64	0.64	0.64	0.64	0.65	0.65
21	0.51	0.52	0.53	0.53	0.55	0.55	0.55	0.56	0.56	0.56	0.56	0.56	0.56	0.57	0.57
22	0.44	0.46	0.46	0.46	0.47	0.48	0.48	0.49	0.48	0.48	0.48	0.48	0.48	0.49	0.49
23	0.37	0.38	0.39	0.39	0.40	0.40	0.40	0.41	0.41	0.41	0.40	0.40	0.40	0.41	0.41
24	0.30	0.31	0.32	0.32	0.32	0.32	0.32	0.33	0.33	0.33	0.32	0.32	0.32	0.33	0.33
25	0.23	0.23	0.24	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.24	0.24	0.24	0.24	0.24
26	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16
27	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
	Add to the Per Cent Sucrose														
29	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
30	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
31	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
32	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.34	0.34	0.34	0.34	0.33	0.33	0.33	0.33
33	0.44	0.44	0.44	0.44	0.44	0.43	0.43	0.43	0.43	0.42	0.42	0.42	0.42	0.42	0.42
34	0.54	0.54	0.53	0.53	0 53	0.52	0 52	0.52	0.52	0.51	0.51	0.50	0.50	0.50	0.50
35	0.63	0.63	0.63	0.62	0.62	0.62	0.61	0.61	0.61	0.60	0.60	0.59	0.59	0.59	0.5
36	0.73	0.73	0.73	0.72	0.72	0.72	0.71	0.70	0.70	0.69	0.69	0.68	0.67	0.67	0.6

*See text, p. 103. Taken from *Proceedings* of the Ninth Session of the International Commission for Uniform Methods of Sugar Analysis, London, 1936 (*Intern. Sugar J.*, 39, 24s).

TABLE 9*

Refractive Indices of Fructose Solutions

[All data computed from weights in air with brass weights. Immersion readings are referable solely to the scale of arbitrary units proposed by Pulfrich (*Z. angew. Chem.*, p. 1186; 1899). According to this scale, 14.5 = 1.33300; 50.0 = 1.34650; and 100.0 = 1.36464]

Per Cent	$n_D^{20°}$	Zeiss Immersion Reading 20°	$n_D^{25°}$	Zeiss Immersion Reading 25°	$\frac{-\Delta n}{\Delta t°}$	Per Cent	$n_D^{20°}$	$n_D^{25°}$	$\frac{-\Delta n}{\Delta t°}$	Per Cent	$n_D^{20°}$	$n_D^{25°}$	$\frac{-\Delta n}{\Delta t°}$
					$\times 10^{-6}$				$\times 10^{-6}$				$\times 10^{-6}$
0	1.33300	14.50	1.33252	13.25	96	32	1.38385	1.38297	175	64	1.4479	1.4467	24
1	3442	18.18	3393	16.90	98	33	8564	8476	177	65	501	489	24
2	3585	21.87	3535	20.58	100	34	8745	8655	180	66	524	512	24
3	3729	25.63	3678	24.29	102	35	8927	8836	183	67	547	535	24
4	3874	29.42	3822	28.05	104	36	9111	9018	185	68	569	557	24
5	4020	33.26	3967	31.87	106	37	9295	9201	188	69	592	580	24
6	4167	37.14	4113	35.71	108	38	9481	9386	190	70	615	602	24
7	4315	41.05	4260	39.61	110	39	9669	9573	192	71	638	625	24
8	4464	45.03	4408	43.53	112	40	9858	9760	195	72	661	648	24
9	4614	49.05	4557	47.53	115	41	1.40048	9949	197	73	684	672	25
10	4765	53.11	4707	51.54	117	42	0239	1.40140	199	74	708	695	25
11	4917	57.19	4857	55.57	119	43	0432	0331	202	75	731	719	25
12	5070	61.32	5009	59.68	121	44	0625	0524	204	76	755	742	25
13	5224	65.51	5162	63.81	123	45	0821	0718	206	77	779	766	25
14	5379	69.75	5316	68.00	125	46	1018	0914	208	78	803	790	25
15	5534	74.03	5470	72.25	127	47	1216	1111	210	79	827	814	25
16	5691	78.36	5626	76.56	129	48	1415	1309	213	80	851	838	25
17	5849	82.75	5783	80.92	132	49	1616	1509	214	81	875	862	25
18	6008	87.17	5942	85.28	135	50	1818	1710	216	82	900	887	25
19	6169	91.64	6102	89.71	137	51	2021	1912	218	83	924	912	25
20	6332	96.14	6262	94.17	139	52	2226	2117	219	84	949	936	25
21	6496	100.72	6425	98.71	142	53	2432	2322	221	85	974	961	25
22	6659	105.37	6588	103.31	146	54	2640	2528	223	86	999	986	25
23	6827	.....	6753	.....	148	55	2848	2736	224	87	1.5024	1.5011	25
24	6996	.....	6921	.....	151	56	3058	2945	226	88	049	036	25
25	7166	.....	7088	.....	154	57	3270	3156	228	89	074	062	25
26	7336	.....	7258	.....	157	58	3482	3368	229	90	100	087	25
27	7506	.....	7426	.....	161	59	3696	3581	231	91	126	113	25
28	7680	.....	7598	.....	164	60	3913	3797	232	92	151	139	25
29	7854	.....	7771	.....	166	61	4130	4014	233	93	177	165	25
30	8030	.....	7945	.....	169	62	4348	4232	235	94	203	191	25
31	8207	.....	8121	.....	172	63	4569	4451	236	95	230	217	25

* See text, p. 103. Taken from Jackson and Mathews, *Bur. Standards J. Research*, 8, 438 (1932). The figures in the columns headed $-\Delta n/\Delta t°$ indicate the decrease in the refractive index for one degree centigrade between 20 and 25°, in units of the sixth or fifth decimal place.

TABLE 10*

For Determining the Percentage of Sucrose in Sugar Solutions From the Readings of the Zeiss Immersion Refractometer at 20° C.[1]

Scale Reading[2] 20° C.	n_D^{20}	Sucrose Per Cent	Scale Reading 20° C.	n_D^{20}	Sucrose Per Cent	Scale Reading 20° C.	n_D^{20}	Sucrose Per Cent
14.47	1.33299	0	45	1.34463	7.91	76	1.35606	15.24
15	3320	0.15	46	4500	8.15	77	5642	15.47
16	3358	0.41	47	4537	8.39	78	5678	15.69
17	3397	0.68	48	4575	8.64	79	5714	15.91
18	3435	0.94	49	4612	8.89	80	5750	16.14
19	3474	1.21	50	4650	9.13	81	5786	16.36
20	3513	1.48	51	4687	9.38	82	5822	16.58
21	3551	1.74	52	4724	9.62	83	5858	16.81
22	3590	2.01	53	4761	9.86	84	5894	17.03
23	3628	2.27	54	4798	10.10	85	5930	17.25
24	3667	2.54	55	4836	10.34	86	5966	17.47
25	3705	2.80	56	4873	10.58	87	6002	17.69
26	3743	3.07	57	4910	10.82	88	6038	17.91
27	3781	3.33	58	4947	11.06	89	6074	18.12
28	3820	3.59	59	4984	11.30	90	6109	18.34
29	3858	3.85	60	5021	11.54	91	6145	18.56
30	3896	4.11	61	5058	11.78	92	6181	18 78
31	3934	4.36	62	5095	12.01	93	6217	19.00
32	3972	4.62	63	5132	12.25	94	6252	19.21
33	4010	4.88	64	5169	12.48	95	6287	19.42
34	4048	5.14	65	5205	12.72	96	6323	19.63
35	4086	5.40	66	5242	12.95	97	6359	19.85
36	4124	5.65	67	5279	13.18	98	6394	20.06
37	4162	5.91	68	5316	13.41	99	6429	20.27
38	4199	6.16	69	5352	13.64	100	6464	20.48
39	4237	6.41	70	5388	13.87	101	6500	20.69
40	4275	6.66	71	5425	14.10	102	6535	20.90
41	4313	6.91	72	5461	14.33	103	6570	21.11
42	4350	7.16	73	5497	14.56	104	6605	21.32
43	4388	7.41	74	5533	14.79	105	6640	21.53
44	4426	7.66	75	5569	15.01			

[1] The values in this table were calculated by J. A. Mathews from the five-place indices of Schönrock as given by Landt, *Z. Ver. deut. Zucker-Ind.*, **83**, 692 (1933).

[2] The scale readings refer only to the scale of arbitrary units proposed by Pulfrich, *Z. angew. Chem.*, p. 1168 (1899). According to this scale 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If the immersion refractometer used is calibrated according to another arbitrary scale, the readings must be converted into refractive indices before this table is used to determine the percentage of sugar.

* See text, p. 120. Taken from " Methods of Analysis, A.O.A.C.," 5th ed., p. 670, 1940.

TABLE 11

Reciprocals of Numbers from 1 to 100

Number	Reciprocal	Number	Reciprocal	Number	Reciprocal	Number	Reciprocal
1	1.0000	26	0.0385	51	0.0196	76	0.0132
2	0.5000	27	0.0370	52	0.0192	77	0.0130
3	0.3333	28	0.0357	53	0.0189	78	0.0128
4	0.2500	29	0.0345	54	0.0185	79	0.0127
5	0.2000	30	0.0333	55	0.0182	80	0.0125
6	0.1667	31	0.0323	56	0.0179	81	0.0123
7	0.1429	32	0.0313	57	0.0175	82	0.0122
8	0.1250	33	0.0303	58	0.0172	83	0.0120
9	0.1111	34	0.0294	59	0.0169	84	0.0119
10	0.1000	35	0.0286	60	0.0167	85	0.0118
11	0.0909	36	0.0278	61	0.0164	86	0.0116
12	0.0833	37	0.0270	62	0.0161	87	0.0115
13	0.0769	38	0.0263	63	0.0159	88	0.0114
14	0.0714	39	0.0256	64	0.0156	89	0.0112
15	0.0667	40	0.0250	65	0.0154	90	0.0111
16	0.0625	41	0.0244	66	0.0152	91	0.0110
17	0.0588	42	0.0238	67	0.0149	92	0.0109
18	0.0555	43	0.0233	68	0.0147	93	0.0108
19	0.0526	44	0.0227	69	0.0145	94	0.0106
20	0.0500	45	0.0222	70	0.0143	95	0.0105
21	0.0476	46	0.0217	71	0.0141	96	0.0104
22	0.0455	47	0.0213	72	0.0139	97	0.0103
23	0.0435	48	0.0208	73	0.0137	98	0.0102
24	0.0417	49	0.0204	74	0.0135	99	0.0101
25	0.0400	50	0.0200	75	0.0133	100	0.0100

TABLE 12*

Lane and Eynon Factors for Determining Invert Sugar, Glucose, Fructose, Maltose, Lactose, and Invert Sugar in the Presence of Sucrose

10 ml. Soxhlet Solution

Titer	Invert Sugar					Glucose	Fructose	Anhydrous Maltose $C_{12}H_{22}O_{11}$	Hydrated Maltose $C_{12}H_{22}O_{11}\cdot H_2O$	Anhydrous Lactose $C_{12}H_{22}O_{11}$	Hydrated Lactose $C_{12}H_{22}O_{11}\cdot H_2O$
	No Sucrose	1 g. Sucrose per 100 ml. Solution	5 g. Sucrose per 100 ml. Solution	10 g. Sucrose per 100 ml. Solution	25 g. Sucrose per 100 ml. Solution						
15	50.5	49.9	47.6	46.1	43.4	49.1	52.2	77.2	81.3	64.9	68.3
16	50.6	50.0	47.6	46.1	43.4	49.2	52.3	77.1	81.2	64.8	68.2
17	50.7	50.1	47.6	46.1	43.4	49.3	52.3	77.0	81.1	64.8	68.2
18	50.8	50.1	47.6	46.1	43.3	49.3	52.4	77.0	81.0	64.7	68.1
19	50.8	50.2	47.6	46.1	43.3	49.4	52.5	76.9	80.9	64.7	68.1
20	50.9	50.2	47.6	46.1	43.2	49.5	52.5	76.8	80.8	64.6	68.0
21	51.0	50.2	47.6	46.1	43.2	49.5	52.6	76.7	80.7	64.6	68.0
22	51.0	50.3	47.6	46.1	43.1	49.6	52.7	76.6	80.6	64.6	68.0
23	51.1	50.3	47.6	46.1	43.0	49.7	52.7	76.5	80.5	64.5	67.9
24	51.2	50.3	47.6	46.1	42.9	49.8	52.8	76.4	80.4	64.5	67.9
25	51.2	50.4	47.6	46.0	42.8	49.8	52.8	76.4	80.4	64.5	67.9
26	51.3	50.4	47.6	46.0	42.8	49.9	52.9	76.3	80.3	64.5	67.9
27	51.4	50.4	47.6	46.0	42.7	49.9	52.9	76.2	80.2	64.4	67.8
28	51.4	50.5	47.7	46.0	42.7	50.0	53.0	76.1	80.1	64.4	67.8
29	51.5	50.5	47.7	46.0	42.6	50.0	53.1	76.0	80.0	64.4	67.8
30	51.5	50.5	47.7	46.0	42.5	50.1	53.2	76.0	80.0	64.4	67.8
31	51.6	50.6	47.7	45.9	42.5	50.2	53.2	75.9	79.9	64.4	67.8
32	51.6	50.6	47.7	45.9	42.4	50.2	53.3	75.9	79.9	64.4	67.8
33	51.7	50.6	47.7	45.9	42.3	50.3	53.3	75.8	79.8	64.4	67.8
34	51.7	50.6	47.7	45.8	42.2	50.3	53.4	75.8	79.8	64.4	67.9
35	51.8	50.7	47.7	45.8	42.2	50.4	53.4	75.7	79.7	64.5	67.9
36	51.8	50.7	47.7	45.8	42.1	50.4	53.5	75.6	79.6	64.5	67.9
37	51.9	50.7	47.7	45.7	42.0	50.5	53.5	75.6	79.6	64.5	67.9
38	51.9	50.7	47.7	45.7	42.0	50.5	53.6	75.5	79.5	64.5	67.9
39	52.0	50.8	47.7	45.7	41.9	50.6	53.6	75.5	79.5	64.5	67.9
40	52.0	50.8	47.7	45.6	41.8	50.6	53.6	75.4	79.4	64.5	67.9
41	52.1	50.8	47.7	45.6	41.8	50.7	53.7	75.4	79.4	64.6	68.0
42	52.1	50.8	47.7	45.6	41.7	50.7	53.7	75.3	79.3	64.6	68.0
43	52.2	50.8	47.7	45.5	41.6	50.8	53.8	75.3	79.3	64.6	68.0
44	52.2	50.9	47.7	45.5	41.5	50.8	53.8	75.2	79.2	64.6	68.0
45	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.2	79.2	64.7	68.1
46	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.1	79.1	64.7	68.1
47	52.4	50.9	47.7	45.3	41.3	51.0	53.9	75.1	79.1	64.8	68.2
48	52.4	50.9	47.7	45.3	41.2	51.0	54.0	75.1	79.1	64.8	68.2
49	52.5	51.0	47.7	45.2	41.1	51.0	54.0	75.0	79.0	64.8	68.2
50	52.5	51.0	47.7	45.2	41.0	51.1	54.0	75.0	79.0	64.9	68.3

* Taken from "Methods of Analysis, A.O.A.C.," 5th ed., p. 683, 1940. See text, pp. 755 and 817.

TABLE 12*A**

Auxiliary Table of Lane and Eynon Factors for Determining Invert Sugar in the Presence of Varying Amounts of Sucrose

10 ml. Soxhlet Solution

Grams Sucrose per 100 ml.	Titer							
	15 ml.	20 ml.	25 ml.	30 ml.	35 ml.	40 ml.	45 ml.	50 ml.
0	50.5	50.9	51.2	51.5	51.8	52.0	52.3	52.5
0.5	50.2	50.5	50.8	51.0	51.2	51.4	51.6	51.7
1	49.9	50.2	50.4	50.5	50.7	50.8	50.9	51.0
2	49.2	49.4	49.5	49.7	49.8	49.8	49.9	50.0
3	48.6	48.7	48.8	48.9	49.0	49.0	49.1	49.2
4	48.1	48.1	48.2	48.3	48.3	48.3	48.4	48.4
5	47.6	47.6	47.6	47.7	47.7	47.7	47.7	47.7
6	47.2	47.2	47.2	47.2	47.2	47.2	47.1	47.1
7	46.9	46.9	46.8	46.8	46.8	46.7	46.6	46.6
8	46.7	46.6	46.5	46.5	46.4	46.3	46.2	46.1
9	46.4	46.3	46.2	46.2	46.1	45.9	45.8	45.6
10	46.1	46.1	46.0	46.0	45.8	45.6	45.4	45.2
12	45.6	45.6	45.5	45.4	45.2	45.0	44.7	44.5
14	45.2	45.1	45.0	44.8	44.6	44.4	44.1	43.8
16	44.8	44.7	44.5	44.3	44.1	43.8	43.5	43.2
18	44.4	44.3	44.1	43.9	43.6	43.3	43.0	42.7
20	44.1	43.9	43.7	43.5	43.2	42.8	42.5	42.2
22	43.8	43.6	43.3	43.1	42.8	42.4	42.0	41.7
24	43.5	43.3	43.0	42.7	42.4	42.0	41.6	41.2
25	43.4	43.2	42.8	42.5	42.2	41.8	41.4	41.0

* Private communication from Messrs. Lane and Eynon. See text, p. 817.

TABLE 13*

Lane and Eynon Factors for Determining Invert Sugar, Glucose, Fructose, Maltose, Lactose, and Invert Sugar in the Presence of Sucrose

25 ml. Soxhlet Solution

Titer	Invert Sugar: No Sucrose	Invert Sugar: 1 g. Sucrose per 100 ml. Solution	Glucose	Fructose	Anhydrous Maltose $C_{12}H_{22}O_{11}$	Hydrated Maltose $C_{12}H_{22}O_{11}\cdot H_2O$	Anhydrous Lactose $C_{12}H_{22}O_{11}$	Hydrated Lactose $C_{12}H_{22}O_{11}\cdot H_2O$
15	123.6	122.6	120.2	127.4	197.8	208.2	163.9	172.5
16	123.6	122.7	120.2	127.4	197.4	207.8	163.5	172.1
17	123.6	122.7	120.2	127.5	197.0	207.4	163.1	171.7
18	123.7	122.7	120.2	127.5	196.7	207.1	162.8	171.4
19	123.7	122.8	120.3	127.6	196.5	206.8	162.5	171.1
20	123.8	122.8	120.3	127.6	196.2	206.5	162.3	170.9
21	123.8	122.8	120.3	127.7	195.8	206.1	162.0	170.6
22	123.9	122.9	120.4	127.7	195.5	205.8	161.8	170.4
23	123.9	122.9	120.4	127.8	195.1	205.4	161.6	170.2
24	124.0	122.9	120.5	127.8	194.8	205.1	161.5	170.0
25	124.0	123.0	120.5	127.9	194.5	204.8	161.4	169.9
26	124.1	123.0	120.6	127.9	194.2	204.4	161.2	169.7
27	124.1	123.0	120.6	128.0	193.9	204.1	161.0	169.5
28	124.2	123.1	120.7	128.0	193.6	203.8	160.8	169.3
29	124.2	123.1	120.7	128.1	193.3	203.5	160.7	169.2
30	124.3	123.1	120.8	128.1	193.0	203.2	160.6	169.0
31	124.3	123.2	120.8	128.1	192.8	202.9	160.5	168.9
32	124.4	123.2	120.8	128.2	192.5	202.6	160.4	168.8
33	124.4	123.2	120.9	128.2	192.2	202.3	160.2	168.6
34	124.5	123.3	120.9	128.3	191.9	202.0	160.1	168.5
35	124.5	123.3	121.0	128.3	191.7	201.8	160.0	168.4
36	124.6	123.3	121.0	128.4	191.4	201.5	159.8	168.2
37	124.6	123.4	121.1	128.4	191.2	201.2	159.7	168.1
38	124.7	123.4	121.2	128.5	191.0	201.0	159.6	168.0
39	124.7	123.4	121.2	128.5	190.8	200.8	159.5	167.9
40	124.8	123.4	121.2	128.6	190.5	200.5	159.4	167.8
41	124.8	123.5	121.3	128.6	190.3	200.3	159.3	167.7
42	124.9	123.5	121.4	128.6	190.1	200.1	159.2	167.6
43	124.9	123.5	121.4	128.7	189.8	199.8	159.2	167.6
44	125.0	123.6	121.5	128.7	189.6	199.6	159.1	167.5
45	125.0	123.6	121.5	128.8	189.4	199.4	159.0	167.4
46	125.1	123.6	121.6	128.8	189.2	199.2	159.0	167.4
47	125.1	123.7	121.6	128.9	189.0	199.0	158.9	167.3
48	125.2	123.7	121.7	128.9	188.9	198.9	158.8	167.2
49	125.2	123.7	121.7	129.0	188.8	198.7	158.8	167.2
50	125.3	123.8	121.8	129.0	188.7	198.6	158.7	167.1

* Taken from " Methods of Analysis, A.O.A.C.," 5th ed., p. 684, 1940. See text, pp. 755 and 817.

TABLE 14*

Main's Table for Determining Invert Sugar in the Presence of Sucrose by the Pot Method

(Using Fehling's solution, Soxhlet's modification; time of heating in boiling water, 5 minutes: indicator, 2 drops of 1 per cent methylene blue in each tube.)

Sucrose, grams per 100 ml.	Between 0 and 1	0	1	1	1	2.5	2.5	5	10
Fehling's solution, ml.	20	10	10	5	2.5	5	2.5	2.5	2.5
Solution Required for Reduction	Grams Invert Sugar per 100 ml.			Percentage of Invert Sugar					
ml.									
15	0.648	0.330	0.325	16.6	8.58	6.60	3.35	1.61	0.742
16	0.608	0.309	0.306	15.6	8.06	6.15	3.12	1.50	0.698
17	0.574	0.290	0.288	14.7	7.60	5.78	2.94	1.41	0.658
18	0.543	0.274	0.272	13.9	7.18	5.46	2.78	1.33	0.620
19	0.514	0.260	0.258	13.2	6.80	5.17	2.63	1.26	0.584
20	0.487	0.248	0.246	12.5	6.45	4.90	2.49	1.19	0.551
21	0.462	0.237	0.235	11.9	6.14	4.66	2.37	1.13	0.520
22	0.440	0.226	0.224	11.4	5.86	4.45	2.26	1.08	0.493
23	0.420	0.216	0.214	10.9	5.60	4.26	2.16	1.03	0.470
24	0.402	0.206	0.205	10.5	5.36	4.08	2.06	0.975	0.450
25	0.387	0.198	0.197	10.1	5.14	3.90	1.98	0.935	0.432
26	0.374	0.190	0.190	9.70	4.94	3.76	1.90	0.902	0.416
27	0.361	0.183	0.183	9.34	4.76	3.62	1.82	0.870	0.400
28	0.349	0.177	0.177	9.00	4.58	3.49	1.75	0.840	0.386
29	0.337	0.171	0.171	8.70	4.42	3.37	1.69	0.812	0.373
30	0.326	0.165	0.165	8.40	4.28	3.26	1.63	0.788	0.361
31	0.316	0.160	0.160	8.15	4.14	3.15	1.57	0.760	0.349
32	0.306	0.155	0.155	7.90	4.01	3.04	1.52	0.735	0.338
33	0.297	0.151	0.151	7.65	3.89	2.95	1.47	0.710	0.328
34	0.288	0.147	0.147	7.45	3.78	2.87	1.43	0.690	0.319
35	0.280	0.143	0.143	7.25	3.68	2.80	1.40	0.670	0.312

* Taken from *Intern. Sugar J.*, **34**, 215 (1932). See text, p. 818.

TABLE 15*

MAIN'S TABLE FOR DETERMINING SMALL QUANTITIES OF INVERT SUGAR IN THE PRESENCE OF SUCROSE BY THE POT METHOD

This table gives percentage of invert sugar on sample.

(Using modified Soxhlet's solution (see p. 818); indicator 2 drops of 1 per cent methylene blue in each tube.)

Sucrose, grams per 100 ml.	5	10	20	20	20	30	20	30
Modified Soxhlet's solution, ml.	4	4	4	2	1	1	1	1
Time of heating in boiling water, minutes	5	5	5	5	5	5	10	10
ml. Solution Required for Reduction								
15	0.832	0.384	0.182	0.0878	0.0390	0.0265	0.0106	0.0085
16	0.779	0.356	0.170	0.0828	0.0381	0.0251	0.0095	0.0071
17	0.740	0.338	0.161	0.0783	0.0368	0.0229	0.0086	0.0060
18	0.704	0.324	0.153	0.0739	0.0348	0.0208	0.0079	0.0052
19	0.670	0.310	0.146	0.0697	0.0323	0.0191	0.0074	0.0045
20	0.638	0.296	0.140	0.0660	0.0299	0.0177	0.0069	0.004
21	0.608	0.283	0.134	0.0628	0.0279	0.0166	0.0065	0.0035
22	0.581	0.270	0.128	0.0600	0.0262	0.0158	0.0061	0.0031
23	0.556	0.256	0.123	0.0575	0.0249	0.0151	0.0058	0.0028
24	0.533	0.243	0.118	0.0553	0.0237	0.0145	0.0055	0.0025
25	0.512	0.232	0.113	0.0530	0.0226	0.0139	0.0052	.....
26	0.493	0.222	0.109	0.0510	0.0216	0.0133	0.0050	0.002
27	0.476	0.215	0.105	0.0491	0.0207	0.0128	0.0047	.....
28	0.459	0.209	0.101	0.0473	0.0198	0.0123	0.0045	0.0015
29	0.444	0.203	0.097	0.0455	0.0190	0.0119	0.0043	.....
30	0.429	0.198	0.0935	0.0438	0.0182	0.0114	0.0041	0.001
31	0.415	0.193	0.090	0.0424	0.0175	0.0109	.....	.....
32	0.402	0.189	0.0865	0.0408	0.0168	0.0105	.....	.....
33	0.390	0.185	0.083	0.0393	0.0162	0.0101	.....	.....
34	0.380	0.181	0.080	0.0380	0.0156	0.0097	.....	.....
35	0.370	0.178	0.0765	0.0369	0.0151	0.0094	.....	.....

* Taken from *Intern. Sugar J.*, 34, 215 (1932). See text, p. 818.

TABLE 16*

ALLIHN'S TABLE FOR DETERMINING GLUCOSE

Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose	Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose	Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose	Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
11	12.4	6.6	51	57.4	26.4	91	102.4	46.4	131	147.5	66.7
12	13.5	7.1	52	58.5	26.9	92	103.6	46.9	132	148.6	67.2
13	14.6	7.6	53	59.7	27.4	93	104.7	47.4	133	149.7	67.7
14	15.8	8.1	54	60.8	27.9	94	105.8	47.9	134	150.9	68.2
15	16.9	8.6	55	61.9	28.4	95	107.0	48.4	135	152.0	68.8
16	18.0	9.0	56	63.0	28.8	96	108.1	48.9	136	153.1	69.3
17	19.1	9.5	57	64.2	29.3	97	109.2	49.4	137	154.2	69.8
18	20.3	10.0	58	65.3	29.8	98	110.3	49.9	138	155.4	70.3
19	21.4	10.5	59	66.4	30.3	99	111.5	50.4	139	156.5	70.8
20	22.5	11.0	60	67.6	30.8	100	112.6	50.9	140	157.6	71.3
21	23.6	11.5	61	68.7	31.3	101	113.7	51.4	141	158.7	71.8
22	24.8	12.0	62	69.8	31.8	102	114.8	51.9	142	159.9	72.3
23	25.9	12.5	63	70.9	32.3	103	116.0	52.4	143	161.0	72.9
24	27.0	13.0	64	72.1	32.8	104	117.1	52.9	144	162.1	73.4
25	28.1	13.5	65	73.2	33.3	105	118.2	53.5	145	163.2	73.9
26	29.3	14.0	66	74.3	33.8	106	119.3	54.0	146	164.4	74.4
27	30.4	14.5	67	75.4	34.3	107	120.5	54.5	147	165.5	74.9
28	31.5	15.0	68	76.6	34.8	108	121.6	55.0	148	166.6	75.5
29	32.7	15.5	69	77.7	35.3	109	122.7	55.5	149	167.7	76.0
30	33.8	16.0	70	78.8	35.8	110	123.8	56.0	150	168.9	76.5
31	34.9	16.5	71	79.9	36.3	111	125.0	56.5	151	170.0	77.0
32	36.0	17.0	72	81.1	36.8	112	126.1	57.0	152	171.1	77.5
33	37.2	17.5	73	82.2	37.3	113	127.2	57.5	153	172.3	78.1
34	38.3	18.0	74	83.3	37.8	114	128.3	58.0	154	173.4	78.6
35	39.4	18.5	75	84.4	38.3	115	129.6	58.6	155	174.5	79.1
36	40.5	18.9	76	85.6	38.8	116	130.6	59.1	156	175.6	79.6
37	41.7	19.4	77	86.7	39.3	117	131.7	59.6	157	176.8	80.1
38	42.8	19.9	78	87.8	39.8	118	132.8	60.1	158	177.9	80.7
39	43.9	20.4	79	88.9	40.3	119	134.0	60.6	159	179.0	81.2
40	45.0	20.9	80	90.1	40.8	120	135.1	61.1	160	180.1	81.7
41	46.2	21.4	81	91.2	41.3	121	136.2	61.6	161	181.3	82.2
42	47.3	21.9	82	92.3	41.8	122	137.4	62.1	162	182.4	82.7
43	48.4	22.4	83	93.4	42.3	123	138.5	62.6	163	183.5	83.3
44	49.5	22.9	84	94.6	42.8	124	139.6	63.1	164	184.6	83.8
45	50.7	23.4	85	95.7	43.4	125	140.7	63.7	165	185.8	84.3
46	51.8	23.9	86	96.8	43.9	126	141.9	64.2	166	186.9	84.8
47	52.9	24.4	87	97.9	44.4	127	143.0	64.7	167	188.0	85.3
48	54.0	24.9	88	99.1	44.9	128	144.1	65.2	168	189.1	85.9
49	55.2	25.4	89	100.2	45.4	129	145.2	65.7	169	190.3	86.4
50	56.3	25.9	90	101.3	45.9	130	146.4	66.2	170	191.4	86.9

* See text, p. 766.

TABLE 16 (*Continued*)

Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose	Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose	Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose	Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
171	192.5	87.4	216	243.2	111.1	261	293.8	135.1	306	344.5	159.8
172	193.6	87.9	217	244.3	111.6	262	295.0	135.7	307	345.6	160.4
173	194.8	88.5	218	245.4	112.1	263	296.1	136.2	308	346.8	160.9
174	195.9	89.0	219	246.6	112.7	264	297.2	136.8	309	347.9	161.5
175	197.0	89.5	220	247.7	113.2	265	298.3	137.3	310	349.0	162.0
176	198.1	90.0	221	248.7	113.7	266	299.5	137.8	311	350.1	162.6
177	199.3	90.5	222	249.9	114.3	267	300.6	138.4	312	351.3	163.1
178	200.4	91.1	223	251.0	114.8	268	301.7	138.9	313	352.4	163.7
179	201.5	91.6	224	252.4	115.3	269	302.8	139.5	314	353.5	164.2
180	202.6	92.1	225	253.3	115.9	270	304.0	140.0	315	354.6	164.8
181	203.8	92.6	226	254.4	116.4	271	305.1	140.6	316	355.8	165.3
182	204.9	93.1	227	255.6	116.9	272	306.2	141.1	317	356.9	165.9
183	206.0	93.7	228	256.7	117.4	273	307.3	141.7	318	358.0	166.4
184	207.1	94.2	229	257.8	118.0	274	308.5	142.2	319	359.1	167.0
185	208.3	94.7	230	258.9	118.5	275	309.6	142.8	320	360.3	167.5
186	209.4	95.2	231	260.1	119.0	276	310.7	143.3	321	361.4	168.1
187	210.5	95.7	232	261.2	119.6	277	311.9	143.9	322	362.5	168.6
188	211.7	96.3	233	262.3	120.1	278	313.0	144.4	323	363.7	169.2
189	212.8	96.8	234	263.4	120.7	279	314.1	145.0	324	364.8	169.7
190	213.9	97.3	235	264.6	121.2	280	315.2	145.5	325	365.9	170.3
191	215.0	97.8	236	265.7	121.7	281	316.4	146.1	326	367.0	170.9
192	216.2	98.4	237	266.8	122.3	282	317.5	146.6	327	368.2	171.4
193	217.3	98.9	238	268.0	122.8	283	318.6	147.2	328	369.3	172.0
194	218.4	99.4	239	269.1	123.4	284	319.7	147.7	329	370.4	172.5
195	219.5	100.0	240	270.2	123.9	285	320.9	148.3	330	371.5	173.1
196	220.7	100.5	241	271.3	124.4	286	322.0	148.8	331	372.7	173.7
197	221.8	101.0	242	272.5	125.0	287	323.1	149.4	332	373.8	174.2
198	222.9	101.5	243	273.6	125.5	288	324.2	149.9	333	374.9	174.8
199	224.0	102.0	244	274.7	126.0	289	325.4	150.5	334	376.0	175.3
200	225.2	102.6	245	275.8	126.6	290	326.5	151.0	335	377.2	175.9
201	226.3	103.1	246	277.0	127.1	291	327.6	151.6	336	378.3	176.5
202	227.4	103.7	247	278.1	127.6	292	328.7	152.1	337	379.4	177.0
203	228.5	104.2	248	279.2	128.1	293	329.9	152.7	338	380.5	177.6
204	229.7	104.7	249	280.3	128.7	294	331.0	153.2	339	381.7	178.1
205	230.8	105.3	250	281.5	129.2	295	332.1	153.8	340	382.8	178.7
206	231.9	105.8	251	282.6	129.7	296	333.3	154.3	341	383.9	179.3
207	233.0	106.3	252	283 7	130.3	297	334.4	154.9	342	385.0	179.8
208	234.2	106.8	253	284.8	130 8	298	335.5	155.4	343	386 2	180.4
209	235.3	107.4	254	286 0	131.4	299	336.6	156.0	344	387.3	180.9
210	236.4	107.9	255	287.1	131.9	300	337.8	156.5	345	388.4	181.5
211	237.6	108.4	256	288 2	132.4	301	338 9	157 1	346	389.6	182.1
212	238.7	109.0	257	289.3	133.0	302	340.0	157 6	347	390.7	182.6
213	239.8	109.5	258	290.5	133.5	303	341.1	158.2	348	391.8	183.2
214	240.9	110.0	259	291.6	134.1	304	342.3	158.7	349	392.9	183.7
215	242.1	110.6	260	292.7	134.6	305	343.4	159.3	350	394.0	184.3

TABLE 16 (*Concluded*)

Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose	Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose	Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose	Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
351	395.2	184.9	380	427.8	201.4	408	459.4	217.5	436	490.9	233.9
352	396.3	185.4	381	429.0	202.0	409	460.5	218.1	437	492.0	234.5
353	397.4	186.0	382	430.1	202.5	410	461.6	218.7	438	493.1	235.1
354	398.6	186.6	383	431.2	203.1	411	462.7	219.3	439	494.3	235.7
355	399.7	187.2	384	432.3	203.7	412	463.8	219.9	440	495.4	236.3
356	400.8	187.7	385	433.5	204.3	413	465.0	220.4	441	496.5	236.9
357	401.9	188.3	386	434.6	204.8	414	466.1	221.0	442	497.6	237.5
358	403.1	188.9	387	435.7	205.4	415	467.2	221.6	443	498.8	238.1
359	404.2	189.4	388	436.8	206.0	416	468.4	222.2	444	499.9	238.7
360	405.3	190.0	389	438.0	206.5	417	469.5	222.8	445	501.0	239.3
361	406.4	190.6	390	439.1	207.1	418	470.6	223.3	446	502.1	239.8
362	407.6	191.1	391	440.2	207.7	419	471.8	223.9	447	503.2	240.4
363	408.7	191.7	392	441.3	208.3	420	472.9	224.5	448	504.4	241.0
364	409.8	192.3	393	442.4	208.8	421	474.0	225.1	449	505.5	241.6
365	410.9	192.9	394	443.6	209.4	422	475.6	225.7	450	506.6	242.2
366	412.1	193.4	395	444.7	210.0	423	476.2	226.3	451	507.8	242.8
367	413.2	194.0	396	445.9	210.6	424	477.4	226.9	452	508.9	243.4
368	414.3	194.6	397	447.0	211.2	425	478.5	227.5	453	510.0	244.0
369	415.4	195.1	398	448.1	211.7	426	479.6	228.0	454	511.1	244.6
370	416.6	195.7	399	449.2	212.3	427	480.7	228.6	455	512.3	245.2
371	417.7	196.3	400	450.3	212.9	428	481.9	229.2	456	513.4	245.7
372	418.8	196.8	401	451.5	213.5	429	483.0	229.8	457	514.5	246.3
373	420.0	197.4	402	452.6	214.1	430	484.1	230.4	458	515.6	246.9
374	421.1	198.0	403	453.7	214.6	431	485.3	231.0	459	516.8	247.5
375	422.2	198.6	404	454.8	215.2	432	486.4	231.6	460	517.9	248.1
376	423.3	199.1	405	456.0	215.8	433	487.5	232.2	461	519.0	248.7
377	424.5	199.7	406	457.1	216.4	434	488.6	232.8	462	520.1	249.3
378	425.6	200.3	407	458.2	217.0	435	489.7	233.4	463	521.3	249.9
379	426.7	200.8									

TABLE 17*

MEISSL'S TABLE FOR DETERMINING INVERT SUGAR

Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
90	46.9	135	70.8	180	95.2	225	120.4
91	47.4	136	71.3	181	95.7	226	120.9
92	47.9	137	71.9	182	96.2	227	121.5
93	48.4	138	72.4	183	96.8	228	122.1
94	48.9	139	72.9	184	97.3	229	122.6
95	49.5	140	73.5	185	97.8	230	123.2
96	50.0	141	74.0	186	98.4	231	123.8
97	50.5	142	74.5	187	99.0	232	124.3
98	51.1	143	75.1	188	99.5	233	124.9
99	51.6	144	75.6	189	100.1	234	125.5
100	52.1	145	76.1	190	100.6	235	126.0
101	52.7	146	76.7	191	101.2	236	126.6
102	53.2	147	77.2	192	101.7	237	127.2
103	53.7	148	77.8	193	102.3	238	127.8
104	54.3	149	78.3	194	102.9	239	128.3
105	54.8	150	78.9	195	103.4	240	128.9
106	55.3	151	79.4	196	104.0	241	129.5
107	55.9	152	80.0	197	104.6	242	130.0
108	56.4	153	80.5	198	105.1	243	130.6
109	56.9	154	81.0	199	105.7	244	131.2
110	57.5	155	81.6	200	106.3	245	131.8
111	58.0	156	82.1	201	106.8	246	132.3
112	58.5	157	82.7	202	107.4	247	132.9
113	59.1	158	83.2	203	107.9	248	133.5
114	59.6	159	83.8	204	108.5	249	134.1
115	60.1	160	84.3	205	109.1	250	134.6
116	60.7	161	84.8	206	109.6	251	135.2
117	61.2	162	85.4	207	110.2	252	135.8
118	61.7	163	85.9	208	110.8	253	136.3
119	62.3	164	86.5	209	111.3	254	136.9
120	62.8	165	87.0	210	111.9	255	137.5
121	63.3	166	87.6	211	112.5	256	138.1
122	63.9	167	88.1	212	113.0	257	138.6
123	64.4	168	88.6	213	113.6	258	139.2
124	64.9	169	89.2	214	114.2	259	139.8
125	65.5	170	89.7	215	114.7	260	140.4
126	66.0	171	90.3	216	115.3	261	140.9
127	66.5	172	90.8	217	115.8	262	141.5
128	67.1	173	91.4	218	116.4	263	142.1
129	67.6	174	91.9	219	117.0	264	142.7
130	68.1	175	92.4	220	117.5	265	143.2
131	68.7	176	93.0	221	118.1	266	143.8
132	69.2	177	93.5	222	118.7	267	144.4
133	69.7	178	94.1	223	119.2	268	144.9
134	70.3	179	94.6	224	119.8	269	145.5

* See text, p. 797.

TABLE 17 (*Concluded*)

Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
270	146.1	310	169.7	350	193.8	390	218.7
271	146.7	311	170.3	351	194.4	391	219.3
272	147.2	312	170.9	352	195.0	392	219.9
273	147.8	313	171.5	353	195.6	393	220.5
274	148.4	314	172.1	354	196.2	394	221.2
275	149.0	315	172.7	355	196.8	395	221.8
276	149.5	316	173.3	356	197.4	396	222.4
277	150.1	317	173.9	357	198.0	397	223.1
278	150.7	318	174.5	358	198.6	398	223.7
279	151.3	319	175.1	359	199.2	399	224.3
280	151.9	320	175.6	360	199.8	400	224.9
281	152.5	321	176.2	361	200.4	401	225.7
282	153.1	322	176.8	362	201.1	402	226.4
283	153.7	323	177.4	363	201.7	403	227.1
284	154.3	324	178.0	364	202.3	404	227.8
285	154.9	325	178.6	365	203.0	405	228.6
286	155.5	326	179.2	366	203.6	406	229.3
287	156.1	327	179.8	367	204.2	407	230.0
288	156.7	328	180.4	368	204.8	408	230.7
289	157.2	329	181.0	369	205.5	409	231.4
290	157.8	330	181.6	370	206.1	410	232.1
291	158.4	331	182.2	371	206.7	411	232.8
292	159.0	332	182.8	372	207.3	412	233.5
293	159.6	333	183.5	373	208.0	413	234.3
294	160.2	334	184.1	374	208.6	414	235.0
295	160.8	335	184.7	375	209.2	415	235.7
296	161.4	336	185.4	376	209.9	416	236.4
297	162.0	337	186.0	377	210.5	417	237.1
298	162.6	338	186.6	378	211.1	418	237.8
299	163.2	339	187.2	379	211.7	419	238.5
300	163.8	340	187.8	380	212.4	420	239.2
301	164.4	341	188.4	381	213.0	421	239.9
302	165.0	342	189.0	382	213.6	422	240.6
303	165.6	343	189.6	383	214.3	423	241.3
304	166.2	344	190.2	384	214.9	424	242.0
305	166.8	345	190.8	385	215.5	425	242.7
306	167.3	346	191.4	386	216.1	426	243.4
307	167.9	347	192.0	387	216.8	427	244.1
308	168.5	348	192.6	388	217.4	428	244.9
309	169.1	349	193.2	389	218.0	429	245.6
						430	246.3

TABLE 18*

Elsdon's Table for Determining Glucose, Fructose, Invert Sugar, Maltose, and Lactose by the Method of Brown, Morris, and Millar

Quantities Expressed in Milligrams in all Cases

Cupric Oxide	Copper	Glucose	Fructose	Lactose Hydrate	Anhydrous Lactose	Anhydrous Maltose	Invert Sugar
100	79.9	.....	.....	59.2	56.2	72.5	.....
101	80.7	.....	.....	59.8	56.8	73.2	.....
102	81.5	.....	.....	60.4	57.3	74.0	.....
103	82.3	.....	.....	61.0	57.9	74.7	.....
104	83.1	.....	.....	61.6	58.5	75.4	.....
105	83.9	.....	.....	62.2	59.1	76.2	.....
106	84.7	.....	.....	62.8	59.6	76.9	.....
107	85.5	.....	.....	63.4	60.2	77.6	.....
108	86.3	.....	.....	64.0	60.8	78.3	.....
109	87.1	.....	.....	64.6	61.3	79.0	44.9
110	87.9	.....	.....	65.2	61.9	79.8	45.3
111	88.7	.....	.....	65.8	62.5	80.5	45.7
112	89.5	.....	.....	66.4	63.0	81.3	46.1
113	90.3	.....	.....	67.0	63.6	82.0	46.5
114	91.1	.....	.....	67.6	64.2	82.7	46.9
115	91.9	.....	49.8	68.2	64.8	83.5	47.3
116	92.7	.....	50.2	68.8	65.3	84.2	47.7
117	93.5	.....	50.6	69.4	65.9	84.9	48.0
118	94.3	.....	50.9	70.0	66.5	85.7	48.4
119	95.1	.....	51.3	70.6	67.0	86.4	48.8
120	95.9	46.5	51.7	71.2	67.6	87.2	49.2
121	96.7	46.9	52.1	71.8	68.2	87.9	49.6
122	97.5	47.3	52.5	72.4	68.7	88.7	50.0
123	98.3	47.7	52.8	73.0	69.3	89.4	50.4
124	99.1	48.1	53.2	73.6	69.9	90.2	50.8
125	99.9	48.5	53.6	74.2	70.5	91.1	51.2
126	100.7	48.8	54.0	74.8	71.0	91.9	51.5
127	101.5	49.2	54.3	75.4	71.6	92.8	51.9
128	102.3	49.6	54.7	76.0	72.2	93.6	52.3
129	103.1	50.0	55.1	76.6	72.7	94.5	52.7
130	103.9	50.4	55.5	77.2	73.3	95.3	53.1
131	104.7	50.8	55.9	77.8	73.9	95.9	53.5
132	105.5	51.2	56.3	78.4	74.4	96.6	53.9
133	106.3	51.5	56.7	79.0	75.0	97.2	54.2
134	107.1	51.9	57.1	79.6	75.6	97.9	54.6
135	107.9	52.3	57.5	80.2	76.2	98.5	55.0
136	108.7	52.7	57.9	80.8	76.7	99.2	55.4
137	109.5	53.1	58.3	81.4	77.3	99.8	55.8
138	110.3	53.5	58.7	82.0	77.9	100.5	56.2
139	111.1	53.8	59.1	82.6	78.4	101.3	56.6

* See text, p. 799. Taken from *Analyst*, 48, 435–443 (1923).

TABLE 18 (*Continued*)

Cupric Oxide	Copper	Glucose	Fructose	Lactose Hydrate	Anhydrous Lactose	Anhydrous Maltose	Invert Sugar
140	111.9	54.2	59.5	83.2	79.0	102.0	57.0
141	112.6	54.6	59.9	83.8	79.6	102.7	57.4
142	113.4	54.9	60.3	84.4	80.2	103.4	57.8
143	114.2	55.3	60.8	85.0	80.7	104.2	58.2
144	115.0	55.7	61.2	85.6	81.3	104.9	58.6
145	115.8	56.0	61.7	86.2	81.9	105.6	59.0
146	116.6	56.4	62.1	86.9	82.4	106.3	59.4
147	117.4	56.8	62.6	87.5	83.0	107.0	59.8
148	118.2	57.2	63.0	88.1	83.6	107.8	60.2
149	119.0	57.6	63.5	88.7	84.2	108.5	60.6
150	119.8	58.0	63.9	89.3	84.8	109.2	61.0
151	120.6	58.3	64.4	90.0	85.4	109.9	61.4
152	121.4	58.7	64.8	90.6	86.0	110.7	61.8
153	122.2	59.1	65.2	91.2	86.5	111.4	62.2
154	123.0	59.5	65.6	91.8	87.1	112.2	62.7
155	123.8	59.9	66.0	92.4	87.7	113.0	63.1
156	124.6	60.3	66.5	93.0	88.3	113.8	63.5
157	125.4	60.7	66.9	93.6	88.8	114.5	63.9
158	126.2	61.1	67.3	94.2	89.4	115.3	64.3
159	127.0	61.5	67.7	94.8	90.0	116.0	64.7
160	127.8	61.8	68.1	95.4	90.6	116.8	65.1
161	128.6	62.2	68.5	96.0	91.2	117.5	65.5
162	129.4	62.6	68.9	96.6	91.7	118.2	65.9
163	130.2	63.0	69.4	97.2	92.3	119.0	66.3
164	131.0	63.4	69.8	97.8	92.9	119.7	66.7
165	131.8	63.8	70.2	98.4	93.5	120.4	67.1
166	132.6	64.2	70.6	99.0	94.0	121.2	67.6
167	133.4	64.5	71.1	99.6	94.6	121.9	68.0
168	134.2	64.9	71.5	100.2	95.2	122.7	68.4
169	135.0	65.3	71.9	100.8	95.8	123.4	68.8
170	135.8	65.7	72.4	101.4	96.3	124.2	69.2
171	136.6	66.1	72.8	102.0	96.9	124.9	69.6
172	137.4	66.5	73.3	102.6	97.4	125.6	70.0
173	138.2	66.9	73.7	103.2	98.0	126.4	70.4
174	139.0	67.3	74.1	103.8	98.6	127.1	70.8
175	139.8	67.6	74.6	104.4	99.2	127.9	71.2
176	140.6	68.0	75.0	105.0	99.7	128.6	71.7
177	141.4	68.4	75.4	105.6	100.3	129.4	72.1
178	142.2	68.8	75.8	106.2	100.9	130.1	72.5
179	143.0	69.2	76.3	106.8	101.5	130.8	72.9
180	143.8	69.6	76.7	107.4	102.0	131.5	73.4
181	144.6	70.0	77.1	108.0	102.6	132.2	73.8
182	145.4	70.4	77.5	108.6	103.2	133.0	74.2
183	146.2	70.8	77.9	109.2	103.8	133.7	74.6
184	147.0	71.2	78.3	109.8	104.3	134.4	75.0

TABLE 18 (*Continued*)

Cupric Oxide	Copper	Glucose	Fructose	Lactose Hydrate	Anhydrous Lactose	Anhydrous Maltose	Invert Sugar
185	147.8	71.6	78.8	110.4	104.9	135.1	75.4
186	148.6	72.0	79.2	111.0	105.5	135.8	75.8
187	149.4	72.4	79.5	111.6	106.1	136.6	76.2
188	150.2	72.8	80.0	112.2	106.7	137.3	76.6
189	151.0	73.2	80.4	112.8	107.3	138.1	77.0
190	151.8	73.6	80.9	113.5	107.9	138.8	77.5
191	152.6	74.0	81.3	114.1	108.5	139.6	77.9
192	153.4	74.4	81.8	114.7	109.1	140.3	78.3
193	154.2	74.8	82.2	115.4	109.6	141.1	78.7
194	155.0	75.2	82.7	116.0	110.2	141.8	79.1
195	155.8	75.6	83.1	116.6	110.8	142.6	79.5
196	156.6	76.0	83.6	117.2	111.3	143.3	79.9
197	157.4	76.4	84.0	117.8	111.9	144.1	80.3
198	158.2	76.8	84.5	118.4	112.5	144.8	80.7
199	159.0	77.2	84.9	119.1	113.1	145.5	81.1
200	159.8	77.6	85.3	119.7	113.7	146.3	81.5
201	160.6	78.0	85.7	120.3	114.3	147.0	81.9
202	161.4	78.4	86.1	121.0	114.9	147.7	82.4
203	162.2	78.8	86.6	121.6	115.5	148.4	82.8
204	163.0	79.2	87.0	122.2	116.1	149.2	83.2
205	163.8	79.6	87.4	122.8	116.7	149.9	83.6
206	164.6	80.0	87.8	123.4	117.3	150.6	84.0
207	165.4	80.4	88.2	124.1	117.9	151.4	84.4
208	166.2	80.8	88.7	124.7	118.5	152.1	84.8
209	167.0	81.2	89.1	125.4	119.1	152.9	85.2
210	167.8	81.6	89.5	126.0	119.7	153.6	85.7
211	168.6	82.0	89.9	126.6	120.3	154.4	86.1
212	169.4	82.4	90.4	127.2	120.9	155.1	86.5
213	170.2	82.7	90.8	127.8	121.5	155.8	87.0
214	171.0	83.1	91.3	128.5	122.1	156.6	87.4
215	171.8	83.5	91.7	129.1	122.6	157.3	87.8
216	172.6	83.9	92.2	129.7	123.2	158.1	88.3
217	173.4	84.3	92.6	130.3	123.8	158.8	88.7
218	174.2	84.7	93.1	130.9	124.4	159.6	89.2
219	175.0	85.1	93.5	131.6	125.0	160.3	89.6
220	175.8	85.5	94.0	132.2	125.6	161.0	90.0
221	176.6	86.0	94.4	132.8	126.2	161.8	90.4
222	177.4	86.4	94.9	133.4	126.8	162.5	90.9
223	178.2	86.9	95.4	134.0	127.4	163.2	91.3
224	179.0	87.3	95.8	134.7	127.9	163.9	91.8
225	179.8	87.8	96.3	135.3	128.5	164.7	92.2
226	180.6	88.2	96.8	135.9	129.1	165.4	92.6
227	181.4	88.7	97.2	136.5	129.7	166.2	93.1
228	182.2	89.1	97.7	137.2	130.3	166.9	93.5
229	183.0	89.6	98.1	137.8	130.9	167.6	93.9

TABLE 18 (*Continued*)

Cupric Oxide	Copper	Glucose	Fructose	Lactose Hydrate	Anhydrous Lactose	Anhydrous Maltose	Invert Sugar
230	183.8	90.0	98.6	138.4	131.5	168.3	94.4
231	184.6	90.4	99.1	139.0	132.1	169.1	94.8
232	185.4	90.8	99.5	139.6	132.7	169.8	95.2
233	186.2	91.2	100.0	140.2	133.3	170.5	95.6
234	187.0	91.7	100.4	140.9	133.9	171.3	96.1
235	187.7	92.1	100.9	141.5	134.4	172.0	96.5
236	188.5	92.5	101.3	142.1	135.0	172.8	97.0
237	189.3	92.9	101.7	142.7	135.6	173.5	97.4
238	190.1	93.4	102.1	143.3	136.2	174.3	97.8
239	190.9	93.8	102.6	144.0	136.8	175.0	98.3
240	191.7	94.2	103.0	144.6	137.4	175.7	98.7
241	192.5	94.6	103.4	145.2	138.0	176.5	99.2
242	193.3	95.0	103.8	145.8	138.6	177.2	99.6
243	194.1	95.4	104.3	146.4	139.1	177.9	100.0
244	194.9	95.8	104.7	147.0	139.7	178.6	100.4
245	195.7	96.2	105.1	147.6	140.3	179.3	100.8
246	196.5	96.6	105.5	148.2	140.8	180.1	101.2
247	197.3	97.0	106.0	148.9	141.4	180.8	101.7
248	198.1	97.5	106.4	149.5	142.0	181.6	102.1
249	198.9	97.9	106.9	150.1	142.6	182.3	102.5
250	199.7	98.3	107.3	150.7	143.2	183.1	102.9
251	200.5	98.7	107.8	151.4	143.8	183.8	103.3
252	201.3	99.1	108.2	152.0	144.4	184.5	103.8
253	202.1	99.5	108.7	152.6	145.0	185.3	104.2
254	202.9	99.9	109.1	153.2	145.6	186.1	104.6
255	203.7	100.3	109.6	153.8	146.2	186.8	105.0
256	204.5	100.8	110.0	154.4	146.8	187.6	105.4
257	205.3	101.2	110.5	155.0	147.4	188.3	105.8
258	206.1	101.6	110.9	155.7	148.0	189.1	106.3
259	206.9	102.1	111.4	156.4	148.6	189.8	106.7
260	207.7	102.5	111.8	157.0	149.2	190.5	107.1
261	208.5	102.9	112.3	157.6	149.8	191.3	107.5
262	209.3	103.4	112.7	158.2	150.3	192.0	108.0
263	210.1	103.8	113.2	158.8	150.9	192.7	108.4
264	210.9	104.2	113.6	159.4	151.5	193.4	108.8
265	211.7	104.7	114.1	160.0	152.1	194.2	109.2
266	212.5	105.1	114.5	160.6	152.6	194.9	109.7
267	213.3	105.5	115.0	161.2	153.2	195.6	110.1
268	214.1	105.9	115.5	161.8	153.8	196.4	110.5
269	214.9	106.3	115.9	162.4	154.4	197.1	111.0
270	215.7	106.7	116.4	163.0	154.9	197.9	111.4
271	216.5	107.1	116.9	163.7	155.5	198.6	111.8
272	217.3	107.6	117.3	164.4	156.1	199.4	112.3
273	218.1	108.0	117.8	165.0	156.6	200.1	112.7
274	218.9	108.4	118.2	165.6	157.2	200.8	113.1

TABLE 18 (*Continued*)

Cupric Oxide	Copper	Glucose	Fructose	Lactose Hydrate	Anhydrous Lactose	Anhydrous Maltose	Invert Sugar
275	219.7	108.8	118.7	166.2	157.8	201.6	113.6
276	220.5	109.2	119.2	166.8	158.4	202.3	114.0
277	221.3	109.6	119.6	167.4	158.9	203.0	114.4
278	222.1	110.0	120.1	168.0	159.5	203.7	114.8
279	222.9	110.4	120.6	168.6	160.1	204.5	115.2
280	223.7	110.8	121.0	169.2	160.7	205.2	115.7
281	224.5	111.2	121.5	169.9	161.3	205.9	116.1
282	225.3	111.7	121.9	170.5	161.9	206.7	116.5
283	226.1	112.1	122.4	171.1	162.5	207.4	117.0
284	226.9	112.5	122.8	171.7	163.1	208.2	117.5
285	227.7	112.9	123.2	172.3	163.7	208.9	117.9
286	228.5	113.4	123.7	173.0	164.3	209.7	118.3
287	229.3	113.8	124.1	173.6	164.9	210.4	118.8
288	230.1	114.2	124.6	174.2	165.5	211.1	119.2
289	230.9	114.6	125.1	174.8	166.1	211.9	119.6
290	231.7	115.0	125.6	175.5	166.7	212.6	120.1
291	232.5	115.4	126.0	176.1	167.4	213.3	120.5
292	233.3	115.9	126.5	176.7	168.0	214.1	121.0
293	234.1	116.3	126.9	177.6	168.7	214.8	121.4
294	234.9	116.7	127.4	178.4	169.3	215.5	121.9
295	235.7	117.2	127.8	179.0	170.0	216.3	122.3
296	236.5	117.6	128.3	179.8	170.6	217.0	122.8
297	237.3	118.1	128.7	180.4	171.3	217.8	123.2
298	238.1	118.5	129.2	181.0	171.9	218.5	123.7
299	238.9	118.9	129.6	181.7	172.6	219.3	124.1
300	239.7	119.4	130.1	182.3	173.2	220.0	124.6
301	240.5	119.8	130.6	183.0	173.8	220.7	125.0
302	241.3	120.2	131.0	183.6	174.5	221.5	125.5
303	242.1	120.6	131.5	184.3	175.1	222.2	125.9
304	242.9	121.1	132.0	185.0	175.7	222.9	126.4
305	243.7	121.5	132.4	185.6	176.4	223.6	126.8
306	244.5	121.9	132.9	186.2	177.0	224.4	127.3
307	245.3	122.2	133.3	186.9	177.6	225.1	127.7
308	246.1	122.8	133.8	187.6	178.3	225.8	128.2
309	246.9	123.2	134.3	188.2	178.9	226.6	128.6
310	247.7	123.7	134.7	188.9	179.5	227.3	129.1
311	248.5	124.1	135.2	189.6	180.1	228.1	129.6
312	249.3	124.5	135.7	190.2	180.7	228.8	130.0
313	250.1	125.0	136.2	190.8	181.3	229.6	130.5
314	250.9	125.5	136.7	191.4	181.9	230.3	130.9
315	251.7	125.9	137.2	192.2	182.6	231.0	131.4
316	252.5	126.4	137.6	192.8	183.2	231.8	131.9
317	253.3	126.8	138.1	193.4	183.8	232.5	132.3
318	254.1	127.3	138.6	194.1	184.4	233.2	132.8
319	254.9	127.7	139.0	194.8	185.0	234.0	133.2

TABLE 18 (*Continued*)

Cupric Oxide	Copper	Glucose	Fructose	Lactose Hydrate	Anhydrous Lactose	Anhydrous Maltose	Invert Sugar
320	255.7	128.2	139.5	195.4	185.6	234.7	133.7
321	256.5	128.6	140.0	196.0	186.2	235.4	134.2
322	257.3	129.1	140.5	196.7	186.9	236.2	134.6
323	258.1	129.5	141.0	197.4	187.5	236.9	135.1
324	258.9	130.0	141.5	198.0	188.1	237.7	135.5
325	259.6	130.4	142.0	198.7	188.7	238.4	136.0
326	260.4	130.9	142.5	199.3	189.4	239.2	136.4
327	261.2	131.3	143.0	200.0	190.0	239.9	136.9
328	262.0	131.8	143.5	200.7	190.7	240.6	137.3
329	262.8	132.2	144.0	201.4	191.3	241.4	137.8
330	263.6	132.6	144.5	202.0	191.9	242.1	138.2
331	264.4	133.1	145.0	202.7	192.6	242.8	138.7
332	265.2	133.5	145.5	203.4	193.2	243.5	139.1
333	266.0	133.9	146.0	204.0	193.9	244.3	139.6
334	266.8	134.4	146.5	204.7	194.5	245.0	140.0
335	267.6	134.8	147.0	205.4	195.1	245.7	140.4
336	268.4	135.3	147.5	206.0	195.8	246.5	140.9
337	269.2	135.7	148.0	206.7	196.4	247.2	141.4
338	270.0	136.2	148.5	207.4	197.1	248.0	141.9
339	270.8	136.7	149.0	208.0	197.7	248.7	142.3
340	271.6	137.1	149.5	208.7	198.3	249.5	142.8
341	272.4	137.6	150.0	209.4	198.9	250.2	143.2
342	273.2	138.0	150.4	210.0	199.5	250.9	143.7
343	274.0	138.5	150.9	210.7	200.2	251.7	144.2
344	274.8	139.0	151.4	211.3	200.8	252.4	144.7
345	275.6	139.4	151.9	212.0	201.4	253.2	145.1
346	276.4	139.9	152.3	212.6	202.0	253.9	145.6
347	277.2	140.4	152.8	213.3	202.7	254.7	146.1
348	278.0	140.8	153.2	214.0	203.3	255.4	146.6
349	278.8	141.3	153.7	214.6	203.9	256.1	147.2
350	279.6	141.7	154.2	215.3	204.5	256.9	147.7
351	280.4	142.2	154.6	216.0	205.1	257.6	148.2
352	281.2	142.6	155.1	216.6	205.8	258.3	148.7
353	282.0	143.1	155.6	217.3	206.4	259.1	149.2
354	282.8	143.5	156.1	218.0	207.1	259.8	149.7
355	283.6	144.0	156.5	218.7	207.7	260.5	150.2
356	284.4	144.5	157.0	219.4	208.3	261.3	150.7
357	285.2	144.9	157.5	220.0	209.0	262.0	151.2
358	286.0	145.4	158.0	220.7	209.6	262.8	151.7
359	286.8	145.9	158.5	221.3	210.3	263.5	152.2
360	287.6	146.4	158.9	222.0	210.9	264.3	152.7
361	288.4	146.9	159.4	222.6	211.5	265.0	153.2
362	289.2	147.4	159.9	223.4	212.1	265.7	153.7
363	290.0	147.9	160.4	224.0	212.8	266.5	154.2
364	290.8	148.4	160.9	224.6	213.4	267.2	154.7

TABLE 18 (*Continued*)

Cupric Oxide	Copper	Glucose	Fructose	Lactose Hydrate	Anhydrous Lactose	Anhydrous Maltose	Invert Sugar
365	291.6	148.9	161.3	225.3	214.0	267.9	155.2
366	292.4	149.4	161.8	226.0	214.7	268.6	155.7
367	293.2	149.9	162.3	226.6	215.3	269.4	156.1
368	294.0	150.3	162.8	227.3	216.0	270.1	156.6
369	294.8	150.8	163.3	228.0	216.6	270.8	157.0
370	295.6	151.2	163.8	228.6	217.2	271.6	157.5
371	296.4	151.6	164.2	229.3	217.8	272.3	157.9
372	297.2	152.1	164.7	230.0	218.4	273.0	158.4
373	298.0	152.5	165.2	230.6	219.1	273.7	158.8
374	298.8	152.9	165.7	231.3	219.7	274.5	159.3
375	299.6	153.4	166.1	232.0	220.3	275.2	159.8
376	300.4	153.8	166.6	232.6	220.9	275.9	160.2
377	301.2	154.3	167.1	233.2	221.6	276.7	160.6
378	302.0	154.7	167.6	234.0	222.2	277.5	161.1
379	302.8	155.1	168.0	234.6	222.8	278.2	161.5
380	303.6	155.6	168.5	235.2	223.4	279.0	161.9
381	304.4	156.1	169.0	235.9	224.0	279.7	162.4
382	305.2	156.6	169.4	236.6	224.7	280.4	162.8
383	306.0	157.1	169.9	237.2	225.3	281.2	163.3
384	306.8	157.5	170.4	237.9	225.9	281.9	163.7
385	307.6	158.0	170.9	238.6	226.6	282.7	164.1
386	308.4	158.5	171.4	239.2	227.2	283.4	164.6
387	309.2	159.0	171.9	239.9	227.9	284.2	165.0
388	310.0	159.5	172.5	240.6	228.5	284.9	165.5
389	310.8	160.0	173.0	241.2	229.2	285.6	166.0
390	311.6	160.5	173.5	241.9	229.8	286.4	166.5
391	312.4	161.0	174.0	242.6	230.4	287.1	167.0
392	313.2	161.4	174.5	243.2	231.1	287.8	167.5
393	314.0	161.9	175.0	243.9	231.7	288.5	168.0
394	314.8	162.3	175.5	244.6	232.4	289.3	168.5
395	315.6	162.8	176.0	245.2	233.0	290.0	169.0
396	316.4	163.3	176.5	245.9	233.6	290.7	169.5
397	317.2	163.7	177.0	246.6	234.3	291.5	170.0
398	318.0	164.2	177.4	247.2	234.9	292.2	170.5
399	318.8	164.7	177.9	247.8	235.6	293.0	170.9
400	319.6	165.2	178.4	248.6	236.2	293.7	171.4
401	320.4	165.7	178.9	249.2	236.8	294.5	171.9
402	321.2	166.2	179.4	249.8	237.4	295.2	172.4
403	322.0	166.7	179.9	250.5	237.9	295.9	172.8
404	322.8	167.2	180.4	251.1	238.5	296.7	173.3
405	323.6	167.6	180.9	251.7	239.1	297.4	173.8
406	324.4	168.1	181.4	252.3	239.6	298.2	174.3
407	325.2	168.6	181.9	252.9	240.2	298.9	174.7
408	326.0	169.1	182.4	253.5	240.8	299.7	175.2
409	326.8	169.6	182.8	254.1	241.4	300.4	175.7
410	327.6	170.1	183.3	254.7	242.0	301.1	176.2
411	328.4	170.6	183.8	255.4	242.6	301.8	176.7
412	329.2	171.1	184.3	256.0	243.2	302.6	177.2
413	330.0	171.6	184.8	256.6	243.8	303.3	177.7
414	330.8	172.1	185.3	257.2	244.4	304.0	178.2

TABLE 18 (*Concluded*)

Cupric Oxide	Copper	Glucose	Fructose	Lactose Hydrate	Anhydrous Lactose	Anhydrous Maltose	Invert Sugar
415	331.5	172.5	185.8	257.8	244.9	304.7	178.7
416	332.3	173.0	186.3	258.4	245.5	.....	179.2
417	333.1	173.5	186.8	259.0	246.1	.....	179.7
418	333.9	174.0	187.3	259.6	246.7	.....	180.2
419	334.7	174.5	187.8	260.2	247.3	.....	180.7
420	335.5	175.0	188.3	260.9	247.9	.....	181.2
421	336.3	175.5	188.8	261.5	248.5	.....	181.7
422	337.1	176.0	189.3	262.1	249.1	.....	182.2
423	337.9	176.5	189.8	262.8	249.7	.....	182.7
424	338.7	177.0	190.3	263.4	250.2	.....	183.2
425	339.5	177.4	190.8	264.0	250.8	.....	183.7
426	340.3	177.9	191.3	264.6	251.4	.....	184.2
427	341.1	178.4	191.8	265.4	252.0	.....	184.7
428	341.9	178.9	192.3	266.0	252.6	.....	185.2
429	342.7	179.4	192.7	266.6	253.2	.....	185.7
430	343.5	179.9	193.2	267.2	253.8	.....	186.2
431	344.3	180.4	193.7	267.9	254.4	.....	186.7
432	345.1	180.9	194.2	268.6	255.1	.....	187.2
433	345.9	181.4	194.7	269.2	255.7	.....	187.7
434	346.7	181.9	195.2	269.9	256.3	.....	188.2
435	347.5	182.5	195.7	270.5	257.0	.....	188.7
436	348.3	183.0	196.3	271.2	257.6	.....	189.2
437	349.1	183.5	196.8	271.8	258.2	.....	189.7
438	349.9	184.0	197.3	272.4	258.9	.....	190.2
439	350.7	184.5	197.8	273.1	259.5	.....	190.7
440	351.5	185.0	198.4	273.8	260.1	.....	191.2
441	352.3	185.5	198.9	274.4	260.7	.....	191.7
442	353.1	186.0	199.5	275.1	261.4	.....	192.2
443	353.9	186.5	200.0	275.8	262.0	.....	192.7
444	354.7	187.0	200.5	276.4	262.6	.....	193.2
445	355.5	187.5	201.1	277.0	263.3	.....	193.7
446	356.3	188.0	201.6	277.7	263.9	.....	194.2
447	357.1	188.5	202.1	278.4	264.5	.....	194.7
448	357.9	189.0	202.6	279.0	265.2	.....	195.2
449	358.7	189.5	203.1	279.7	265.8	.....	195.7
450	359.5	190.0	203.6	280.4	266.4	.....	196.3
451	360.3	190.5	204.2	281.0	267.0	.....	196.8
452	361.1	191.0	204.7	281.7	267.7	.....	197.3
453	361.9	191.5	205.2	282.4	268.3	.....	197.9
454	362.7	192.0	205.7	283.0	269.0	.....	198.4
455	363.5	192.5	206.2	283.7	269.6	.....	198.9
456	364.3	193.0	206.8	284.4	270.2	.....	199.5
457	365.1	193.5	207.3	285.1	270.9	.....	200.0
458	365.9	194.0	207.8	285.8	271.5	.....	200.5
459	366.7	194.5	208.3	286.5	272.2	.....	201.1
460	367.5	195.0	208.8	287.2	272.8	.....	201.6

TABLE 19A*

MUNSON AND WALKER'S TABLE FOR DETERMINING GLUCOSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 g. AND 2 g. TOTAL SUGAR), LACTOSE, LACTOSE AND SUCROSE (2 MIXTURES), AND MALTOSE (CRYSTALLIZED)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
10	8.9	4.0	4.5	1.6	.....	6.3	6.1	.....	6.2	10
11	9.8	4.5	5.0	2.1	.....	6.9	6.7	.....	7.0	11
12	10.7	4.9	5.4	2.5	.....	7.5	7.3	.....	7.9	12
13	11.5	5.3	5.8	3.0	.....	8.2	7.9	.....	8.7	13
14	12.4	5.7	6.3	3.4	.....	8.8	8.5	.....	9.5	14
15	13.3	6.2	6.7	3.9	.....	9.4	9.1	.....	10.4	15
16	14.2	6.6	7.2	4.3	.....	10.0	9.7	.....	11.2	16
17	15.1	7.0	7.6	4.8	.....	10.7	10.3	.....	12.0	17
18	16.0	7.5	8.1	5.2	.....	11.3	10.9	.....	12.9	18
19	16.9	7.9	8.5	5.7	.....	11.9	11.5	.....	13.7	19
20	17.8	8.3	8.9	6.1	.....	12.5	12.1	.....	14.6	20
21	18.7	8.7	9.4	6.6	.....	13.2	12.7	.....	15.4	21
22	19.5	9.2	9.8	7.0	.....	13.8	13.3	.....	16.2	22
23	20.4	9.6	10.3	7.5	.....	14.4	13.9	.....	17.1	23
24	21.3	10.0	10.7	7.9	.....	15.0	14.5	.....	17.9	24
25	22.2	10.5	11.2	8.4	.....	15.7	15.2	.....	18.7	25
26	23.1	10.9	11.6	8.8	.....	16.3	15.8	.....	19.6	26
27	24.0	11.3	12.0	9.3	.....	16.9	16.4	.....	20.4	27
28	24.9	11.8	12.5	9.7	.....	17.6	17.0	.....	21.2	28
29	25.8	12.2	12.9	10.2	.....	18.2	17.6	.....	22.1	29
30	26.6	12.6	13.4	10.7	4.3	18.8	18.2	.....	22.9	30
31	27.5	13.1	13.8	11.1	4.7	19.4	18.8	.....	23.7	31
32	28.4	13.5	14.3	11.6	5.2	20.1	19.4	.....	24.6	32
33	29.3	13.9	14.7	12.0	5.6	20.7	20.0	.....	25.4	33
34	30.2	14.3	15.2	12.5	6.1	21.4	20.7	.....	26.2	34
35	31.1	14.8	15.6	12.9	6.5	22.1	21.3	.....	27.1	35
36	32.0	15.2	16.1	13.4	7.0	22.8	22.0	.....	27.9	36
37	32.9	15.6	16.5	13.8	7.4	23.5	22.7	.....	28.7	37
38	33.8	16.1	16.9	14.3	7.9	24.2	23.3	.....	29.6	38
39	34.6	16.5	17.4	14.7	8.4	24.8	24.0	.....	30.4	39
40	35.5	16.9	17.8	15.2	8.8	25.5	24.7	.....	31.3	40
41	36.4	17.4	18.3	15.6	9.3	26.2	25.3	.....	32.1	41
42	37.3	17.8	18.7	16.1	9.7	26.9	26.0	.....	32.9	42
43	38.2	18.2	19.2	16.6	10.2	27.6	26.6	.....	33.8	43
44	39.1	18.7	19.6	17.0	10.7	28.3	27.3	.....	34.6	44
45	40.0	19.1	20.1	17.5	11.1	28.9	28.0	.....	35.4	45
46	40.9	19.6	20.5	17.9	11.6	29.6	28.6	.....	36.3	46
47	41.7	20.0	21.0	18.4	12.0	30.3	29.3	.....	37.1	47
48	42.6	20.4	21.4	18.8	12.5	31.0	30.0	.....	37.9	48
49	43.5	20.9	21.9	19.3	12.9	31.7	30.6	.....	38.8	49

* Taken from "Methods of Analysis, A.O.A.C.," 2nd ed., pp. 434–445, 1925. See text, pp. 801 and 812.

TABLE 19*A* (*Continued*)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
50	44.4	21.3	22.3	19.7	13.4	32.3	31.3	.....	39.6	50
51	45.3	21.7	22.8	20.2	13.9	33.0	32.0	.....	40.4	51
52	46.2	22.2	23.2	20.7	14.3	33.7	32.6	.....	41.3	52
53	47.1	22.6	23.7	21.1	14.8	34.4	33.3	.....	42.1	53
54	48.0	23.0	24.1	21.6	15.2	35.1	34.0	.....	42.9	54
55	48.9	23.5	24.6	22.0	15.7	35.8	34.6	.....	43.8	55
56	49.7	23.9	25.0	22.5	16.2	36.4	35.3	.....	44.6	56
57	50.6	24.3	25.5	22.9	16.6	37.1	35.9	.....	45.4	57
58	51.5	24.8	25.9	23.4	17.1	37.8	36.6	.....	46.3	58
59	52.4	25.2	26.4	23.9	17.5	38.5	37.3	.....	47.1	59
60	53.3	25.6	26.8	24.3	18.0	39.2	37.9	.....	48.0	60
61	54.2	26.1	27.3	24.8	18.5	39.9	38.6	.....	48.8	61
62	55.1	26.5	27.7	25.2	18.9	40.5	39.3	.....	49.6	62
63	56.0	27.0	28.2	25.7	19.4	41.2	39.9	.....	50.5	63
64	56.8	27.4	28.6	26.2	19.8	41.9	40.6	.....	51.3	64
65	57.7	27.8	29.1	26.6	20.3	42.6	41.3	.....	52.1	65
66	58.6	28.3	29.5	27.1	20.8	43.3	41.9	.....	53.0	66
67	59.5	28.7	30.0	27.5	21.2	44.0	42.6	40.1	53.8	67
68	60.4	29.2	30.4	28.0	21.7	44.7	43.3	40.7	54.6	68
69	61.3	29.6	30.9	28.5	22.2	45.3	43.9	41.3	55.5	69
70	62.2	30.0	31.3	28.9	22.6	46.0	44.6	41.9	56.3	70
71	63.1	30.5	31.8	29.4	23.1	46.7	45.3	42.5	57.1	71
72	64.0	30.9	32.3	29.8	23.5	47.4	45.9	43.1	58.0	72
73	64.8	31.4	32.7	30.3	24.0	48.1	46.6	43.7	58.8	73
74	65.7	31.8	33.2	30.8	24.5	48.8	47.3	44.2	59.6	74
75	66.6	32.2	33.6	31.2	24.9	49.4	47.9	44.8	60.5	75
76	67.5	32.7	34.1	31.7	25.4	50.1	48.6	45.4	61.3	76
77	68.4	33.1	34.5	32.1	25.9	50.8	49.3	46.0	62.1	77
78	69.3	33.6	35.0	32.6	26.3	51.5	49.9	46.6	63.0	78
79	70.2	34.0	35.4	33.1	26.8	52.2	50.6	47.2	63.8	79
80	71.1	34.4	35.9	33.5	27.3	52.9	51.3	47.8	64.6	80
81	71.9	34.9	36.3	34.0	27.7	53.6	51.9	48.4	65.5	81
82	72.8	35.3	36.8	34.5	28.2	54.2	52.6	49.0	66.3	82
83	73.7	35.8	37.3	34.9	28.6	54.9	53.3	49.6	67.1	83
84	74.6	36.2	37.7	35.4	29.1	55.6	53.9	50.1	68.0	84
85	75.5	36.7	38.2	35.8	29.6	56.3	54.6	50.7	68.8	85
86	76.4	37.1	38.6	36.3	30.0	57.0	55.3	51.3	69.7	86
87	77.3	37.5	39.1	36.8	30.5	57.7	55.9	51.9	70.5	87
88	78.2	38.0	39.5	37.2	31.0	58.4	56.6	52.5	71.3	88
89	79.1	38.4	40.0	37.7	31.4	59.0	57.3	53.1	72.2	89

TABLE 19A (*Continued*)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
90	79.9	38.9	40.4	38.2	31.9	59.7	57.9	53.7	73.0	90
91	80.8	39.3	40.9	38.6	32.4	60.4	58.6	54.3	73.8	91
92	81.7	39.8	41.4	39.1	32.8	61.1	59.3	54.9	74.7	92
93	82.6	40.2	41.8	39.6	33.3	61.8	59.9	55.5	75.5	93
94	83.5	40.6	42.3	40.0	33.8	62.5	60.6	56.0	76.3	94
95	84.4	41.1	42.7	40.5	34.2	63.2	61.3	56.6	77.2	95
96	85.3	41.5	43.2	41.0	34.7	63.8	61.9	57.2	78.0	96
97	86.2	42.0	43.7	41.4	35.2	64.5	62.6	57.8	78.8	97
98	87.1	42.4	44.1	41.9	35.6	65.2	63.3	58.4	79.7	98
99	87.9	42.9	44.6	42.4	36.1	65.9	63.9	59.0	80.5	99
100	88.8	43.3	45.0	42.8	36.6	66.6	64.6	59.6	81.3	100
101	89.7	43.8	45.5	43.3	37.0	67.3	65.3	60.2	82.2	101
102	90.6	44.2	46.0	43.8	37.5	68.0	66.0	60.8	83.0	102
103	91.5	44.7	46.4	44.2	38.0	68.7	66.6	61.4	83.8	103
104	92.4	45.1	46.9	44.7	38.5	69.3	67.3	62.0	84.7	104
105	93.3	45.5	47.3	45.2	38.9	70.0	68.0	62.6	85.5	105
106	94.2	46.0	47.8	45.6	39.4	70.7	68.6	63.2	86.3	106
107	95.0	46.4	48.3	46.1	39.9	71.4	69.3	63.8	87.2	107
108	95.9	46.9	48.7	46.6	40.3	72.1	70.0	64.4	88.0	108
109	96.8	47.3	49.2	47.0	40.8	72.8	70.6	65.0	88.8	109
110	97.7	47.8	49.6	47.5	41.3	73.5	71.3	65.6	89.7	110
111	98.6	48.2	50.1	48.0	41.7	74.2	72.0	66.1	90.5	111
112	99.5	48.7	50.6	48.4	42.2	74.8	72.6	66.7	91.3	112
113	100.4	49.1	51.0	48.9	42.7	75.5	73.3	67.3	92.2	113
114	101.3	49.6	51.5	49.4	43.2	76.2	74.0	67.9	93.0	114
115	102.2	50.0	51.9	49.8	43.6	76.9	74.6	68.5	93 9	115
116	103.0	50.5	52.4	50.3	44.1	77.6	75.3	69.1	94.7	116
117	103.9	50.9	52.9	50.8	44.6	78.3	76.0	69.7	95.5	117
118	104.8	51.4	53.3	51.2	45.0	79.0	76.7	70.3	96.4	118
119	105.7	51.8	53.8	51.7	45.5	79.6	77.3	70.9	97.2	119
120	106.6	52.3	54.3	52.2	46.0	80.3	78.0	71.5	98.0	120
121	107.5	52.7	54.7	52.7	46.5	81.0	78.7	72.1	98.9	121
122	108.4	53.2	55.2	53.1	46.9	81.7	79.3	72.7	99.7	122
123	109.3	53.6	55.7	53.6	47.4	82.4	80.0	73.3	100.5	123
124	110.1	54.1	56.1	54.1	47.9	83.1	80.7	73.9	101.4	124
125	111.0	54.5	56.6	54.5	48.3	83.8	81 3	74.5	102.2	125
126	111.9	55.0	57.0	55.0	48.8	84.5	82.0	75.1	103.0	126
127	112.8	55.4	57.5	55.5	49.3	85.1	82.7	75.7	103.9	127
128	113.7	55.9	58.0	55.9	49.8	85.8	83.4	76.3	104.7	128
129	114.6	56.3	58.4	56.4	50.2	86.5	84.0	76.9	105.5	129

TABLE 19A (*Continued*)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
130	115.5	56.8	58.9	56.9	50.7	87.2	84.7	77.5	106.4	130
131	116.4	57.2	59.4	57.4	51.2	87.9	85.4	78.1	107.2	131
132	117.3	57.7	59.8	57.8	51.7	88.6	86.0	78.7	108.0	132
133	118.1	58.1	60.3	58.3	52.1	89.3	86.7	79.3	108.9	133
134	119.0	58.6	60.8	58.8	52.6	90.0	87.4	79.7	109.7	134
135	119.9	59.0	61.2	59.3	53.1	90.6	88.1	80.5	110.5	135
136	120.8	59.5	61.7	59.7	53.6	91.3	88.7	81.1	111.4	136
137	121.7	60.0	62.2	60.2	54.0	92.0	89.4	81.7	112.2	137
138	122.6	60.4	62.6	60.7	54.5	92.7	90.1	82.3	113.0	138
139	123.5	60.9	63.1	61.2	55.0	93.4	90.7	82.9	113.9	139
140	124.4	61.3	63.6	61.6	55.5	94.1	91.4	83.5	114.7	140
141	125.2	61.8	64.0	62.1	55.9	94.8	92.1	84.1	115.5	141
142	126.1	62.2	64.5	62.6	56.4	95.5	92.8	84.7	116.4	142
143	127.0	62.7	65.0	63.1	56.9	96.1	93.4	85.3	117.2	143
144	127.9	63.1	65.4	63.5	57.4	96.8	94.1	85.9	118.0	144
145	128.8	63.6	65.9	64.0	57.8	97.5	94.8	86.5	118.9	145
146	129.7	64.0	66.4	64.5	58.3	98.2	95.4	87.1	119.7	146
147	130.6	64.5	66.9	65.0	58.8	98.9	96.1	87.7	120.5	147
148	131.5	65.0	67.3	65.4	59.3	99.6	96.8	88.3	121.4	148
149	132.4	65.4	67.8	65.9	59.7	100.3	97.5	88.9	122.2	149
150	133.2	65.9	68.3	66.4	60.2	101.0	98.1	89.5	123.0	150
151	134.1	66.3	68.7	66.9	60.7	101.6	98.8	90.2	123.9	151
152	135.0	66.8	69.2	67.3	61.2	102.3	99.5	90.8	124.7	152
153	135.9	67.2	69.7	67.8	61.7	103.0	100.1	91.4	125.5	153
154	136.8	67.7	70.1	68.3	62.1	103.7	100.8	92.0	126.4	154
155	137.7	68.2	70.6	68.8	62.6	104.4	101.5	92.6	127.2	155
156	138.6	68.6	71.1	69.2	63.1	105.1	102.2	93.2	128.0	156
157	139.5	69.1	71.6	69.7	63.6	105.8	102.8	93.8	128.9	157
158	140.3	69.5	72.0	70.2	64.1	106.5	103.5	94.4	129.7	158
159	141.2	70.0	72.5	70.7	64.5	107.2	104.2	95.0	130.5	159
160	142.1	70.4	73.0	71.2	65.0	107.9	104.8	95.6	131.4	160
161	143.0	70.9	73.4	71.6	65.5	108.5	105.5	96.2	132.2	161
162	143.9	71.4	73.9	72.1	66.0	109.2	106.2	96.8	133.0	162
163	144.8	71.8	74.4	72.6	66.5	109.9	106.9	97.4	133.9	163
164	145.7	72.3	74.9	73.1	66.9	110.6	107.5	98.0	134.7	164
165	146.6	72.8	75.3	73.6	67.4	111.3	108.2	98.6	135.5	165
166	147.5	73.2	75.8	74.0	67.9	112.0	108.9	99.2	136.4	166
167	148.3	73.7	76.3	74.5	68.4	112.7	109.6	99.8	137.2	167
168	149.2	74.1	76.8	75.0	68.9	113.4	110.2	100.4	138.0	168
169	150.1	74.6	77.2	75.5	69.3	114.1	110.9	101.0	138.9	169

TABLE 19A (*Continued*)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
170	151.0	75.1	77.7	76.0	69.8	114.8	111.6	101.6	139.7	170
171	151.9	75.5	78.2	76.4	70.3	115.4	112.3	102.2	140.5	171
172	152.8	76.0	78.7	76.9	70.8	116.1	112.9	102.8	141.4	172
173	153.7	76.4	79.1	77.4	71.3	116.8	113.6	103.5	142.2	173
174	154.6	76.9	79.6	77.9	71.7	117.5	114.3	104.1	143.0	174
175	155.5	77.4	80.1	78.4	72.2	118.2	114.9	104.7	143.9	175
176	156.3	77.8	80.6	78.8	72.7	118.9	115.6	105.3	144.7	176
177	157.2	78.3	81.0	79.3	73.2	119.6	116.3	105.9	145.5	177
178	158.1	78.8	81.5	79.8	73.7	120.3	117.0	106.5	146.4	178
179	159.0	79.2	82.0	80.3	74.2	121.0	117.6	107.1	147.2	179
180	159.9	79.7	82.5	80.8	74.6	121.6	118.3	107.7	148.0	180
181	160.8	80.1	82.9	81.3	75.1	122.3	119.0	108.3	148.9	181
182	161.7	80.6	83.4	81.7	75.6	123.1	119.7	108.9	149.7	182
183	162.6	81.1	83.9	82.2	76.1	123.7	120.3	109.5	150.5	183
184	163.4	81.5	84.4	82.7	76.6	124.3	121.0	110.1	151.4	184
185	164.3	82.0	84.9	83.2	77.1	125.1	121.7	110.7	152.2	185
186	165.2	82.5	85.3	83.7	77.6	125.8	122.4	111.3	153.0	186
187	166.1	82.9	85.8	84.2	78.0	126.5	123.1	111.9	153.9	187
188	167.0	83.4	86.3	84.6	78.5	127.2	123.7	112.5	154.7	188
189	167.9	83.9	86.8	85.1	79.0	127.9	124.4	113.1	155.5	189
190	168.8	84.3	87.2	85.6	79.5	128.5	125.1	113.8	156.4	190
191	169.7	84.8	87.7	86.1	80.0	129.2	125.8	114.4	157.2	191
192	170.5	85.3	88.2	86.6	80.5	129.9	126.4	115.0	158.0	192
193	171.4	85.7	88.7	87.1	81.0	130.6	127.1	115.6	158.9	193
194	172.3	86.2	89.2	87.6	81.4	131.3	127.8	116.2	159.7	194
195	173.2	86.7	89.6	88.0	81.9	132.0	128.5	116.8	160.5	195
196	174.1	87.1	90.1	88.5	82.4	132.7	129.2	117.4	161.4	196
197	175.0	87.6	90.6	89.0	82.9	133.4	129.8	118.0	162.2	197
198	175.9	88.1	91.1	89.5	83.4	134.1	130.5	118.6	163.0	198
199	176.8	88.5	91.6	90.0	83.9	134.8	131.2	119.2	163.9	199
200	177.7	89.0	92.0	90.5	84.4	135.4	131.9	119.8	164.7	200
201	178.5	89.5	92.5	91.0	84.8	136.1	132.5	120.4	165.5	201
202	179.4	89.9	93.0	91.4	85.3	136.8	133.2	121.0	166.4	202
203	180.3	90.4	93.5	91.9	85.8	137.5	133.9	121.7	167.2	203
204	181.2	90.9	94.0	92.4	86.3	138.2	134.6	122.3	168.0	204
205	182.1	91.4	94.5	92.9	86.8	138.9	135.3	122.9	168.9	205
206	183.0	91.8	94.9	93.4	87.3	139.6	135.9	123.5	169.7	206
207	183.9	92.3	95.4	93.9	87.8	140.3	136.6	124.1	170.5	207
208	184.8	92.8	95.9	94.4	88.3	141.0	137.3	124.7	171.4	208
209	185.6	93.2	96.4	94.9	88.8	141.7	138.0	125.3	172.2	209

TABLE 19*A* (*Continued*)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
210	186.5	93.7	96.9	95.4	89.2	142.3	138.6	126.0	173.0	210
211	187.4	94.2	97.4	95.8	89.7	143.0	139.3	126.6	173.8	211
212	188.3	94.6	97.8	96.3	90.2	143.7	140.0	127.2	174.7	212
213	189.2	95.1	98.3	96.8	90.7	144.4	140.7	127.8	175.5	213
214	190.1	95.6	98.8	97.3	91.2	145.1	141.4	128.4	176.4	214
215	191.0	96.1	99.3	97.8	91.7	145.8	142.0	129.0	177.2	215
216	191.9	96.5	99.8	98.3	92.2	146.5	142.7	129.6	178.0	216
217	192.8	97.0	100.3	98.8	92.7	147.2	143.4	130.2	178.9	217
218	193.6	97.5	100.8	99.3	93.2	147.9	144.1	130.9	179.7	218
219	194.5	98.0	101.2	99.8	93.7	148.6	144.7	131.5	180.5	219
220	195.4	98.4	101.7	100.3	94.2	149.3	145.4	132.1	181.4	220
221	196.3	98.9	102.2	100.8	94.7	150.0	146.1	132.7	182.2	221
222	197.2	99.4	102.7	101.2	95.1	150.7	146.8	133.3	183.0	222
223	198.1	99.9	103.2	101.7	95.6	151.3	147.5	133.9	183.9	223
224	199.0	100.3	103.7	102.2	96.1	152.0	148.1	134.5	184.7	224
225	199.9	100.8	104.2	102.7	96.6	152.7	148.8	135.2	185.5	225
226	200.7	101.3	104.6	103.2	97.1	153.4	149.5	135.8	186.4	226
227	201.6	101.8	105.1	103.7	97.6	154.1	150.2	136.4	187.2	227
228	202.5	102.2	105.6	104.2	98.1	154.8	150.8	137.0	188.0	228
229	203.4	102.7	106.1	104.7	98.6	155.5	151.5	137.6	188.8	229
230	204.3	103.2	106.6	105.2	99.1	156.2	152.2	138.2	189.7	230
231	205.2	103.7	107.1	105.7	99.6	156.9	152.9	138.8	190.5	231
232	206.1	104.1	107.6	106.2	100.1	157.6	153.6	139.4	191.3	232
233	207.0	104.6	108.1	106.7	100.6	158.3	154.2	140.1	192.2	233
234	207.9	105.1	108.6	107.2	101.1	159.0	154.9	140.7	193.0	234
235	208.7	105.6	109.1	107.7	101.6	159.6	155.6	141.3	193.8	235
236	209.6	106.0	109.5	108.2	102.1	160.3	156.3	141.9	194.7	236
237	210.5	106.5	110.0	108.7	102.6	161.0	156.9	142.5	195.5	237
238	211.4	107.0	110.5	109.2	103.1	161.7	157.6	143.2	196.3	238
239	212.3	107.5	111.0	109.6	103.5	162.4	158.3	143.8	197.2	239
240	213.2	108.0	111.5	110.1	104.0	163.1	159.0	144.4	198.0	240
241	214.1	108.4	112.0	110.6	104.5	163.8	159.7	145.0	198.8	241
242	215.0	108.9	112.5	111.1	105.0	164.5	160.3	145.6	199.7	242
243	215.8	109.4	113.0	111.6	105.5	165.2	161.0	146.3	200.5	243
244	216.7	109.9	113.5	112.1	106.0	165.9	161.7	146.9	201.3	244
245	217.6	110.4	114.0	112.6	106.5	166.6	162.4	147.5	202.2	245
246	218.5	110.8	114.5	113.1	107.0	167.3	163.1	148.1	203.0	246
247	219.4	111.3	115.0	113.6	107.5	168.0	163.7	148.7	203.8	247
248	220.3	111.8	115.4	114.1	108.0	168.7	164.4	149.3	204.7	248
249	221.2	112.3	115.9	114.6	108.5	169.4	165.1	150.0	205.5	249

TABLE 19*A* (*Continued*)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
250	222.1	112.8	116.4	115.1	109.0	170.1	165.8	150.6	206.3	250
251	223.0	113.2	116.9	115.6	109.5	170.8	166.5	151.2	207.2	251
252	223.8	113.7	117.4	116.1	110.0	171.5	167.2	151.8	208.0	252
253	224.7	114.2	117.9	116.6	110.5	172.1	167.8	152.4	208.8	253
254	225.6	114.7	118.4	117.1	111.0	172.8	168.5	153.1	209.7	254
255	226.5	115.2	118.9	117.6	111.5	173.5	169.2	153.7	210.5	255
256	227.4	115.7	119.4	118.1	112.0	174.2	169.9	154.3	211.3	256
257	228.3	116.1	119.9	118.6	112.5	174.9	170.6	154.9	212.2	257
258	229.2	116.6	120.4	119.1	113.0	175.6	171.3	155.5	213.0	258
259	230.1	117.1	120.9	119.6	113.5	176.3	171.9	156.2	213.8	259
260	231.0	117.6	121.4	120.1	114.0	177.0	172.6	156.8	214.7	260
261	231.8	118.1	121.9	120.6	114.5	177.7	173.3	157.4	215.5	261
262	232.7	118.6	122.4	121.1	115.0	178.4	174.0	158.0	216.3	262
263	233.6	119.0	122.9	121.6	115.5	179.1	174.7	158.6	217.2	263
264	234.5	119.5	123.4	122.1	116.0	179.8	175.3	159.3	218.0	264
265	235.4	120.0	123.9	122.6	116.5	180.5	176.0	159.9	218.8	265
266	236.3	120.5	124.4	123.1	117.0	181.2	176.7	160.5	219.7	266
267	237.2	121.0	124.9	123.6	117.5	181.9	177.4	161.1	220.5	267
268	238.1	121.5	125.4	124.1	118.0	182.6	178.1	161.8	221.3	268
269	238.9	122.0	125.9	124.6	118.5	183.3	178.8	162.4	222.1	269
270	239.8	122.5	126.4	125.1	119.0	184.0	179.4	163.0	223.0	270
271	240.7	122.9	126.9	125.6	119.5	184.6	180.1	163.6	223.8	271
272	241.6	123.4	127.4	126.2	120.0	185.3	180.8	164.3	224.6	272
273	242.5	123.9	127.9	126.7	120.6	186.0	181.5	164.9	225.5	273
274	243.4	124.4	128.4	127.2	121.1	186.7	182.2	165.5	226.3	274
275	244.3	124.9	128.9	127.7	121.6	187.4	182.9	166.1	227.1	275
276	245.2	125.4	129.4	128.2	122.1	188.1	183.5	166.8	228.0	276
277	246.1	125.9	129.9	128.7	122.6	188.8	184.2	167.4	228.8	277
278	246.9	126.4	130.4	129.2	123.1	189.5	184.9	168.0	229.6	278
279	247.8	126.9	130.9	129.7	123.6	190.2	185.6	168.7	230.5	279
280	248.7	127.3	131.4	130.2	124.1	190.9	186.3	169.3	231.3	280
281	249.6	127.8	131.9	130.7	124.6	191.6	187.0	169.9	232.1	281
282	250.5	128.3	132.4	131.2	125.1	192.3	187.6	170.5	233.0	282
283	251.4	128.8	132.9	131.7	125.6	193.0	188.3	171.2	233.8	283
284	252.3	129.3	133.4	132.2	126.1	193.7	189.0	171.8	234.6	284
285	253.2	129.8	133.9	132 7	126.6	194 4	189.7	172 4	235.5	285
286	254.0	130.3	134.4	133.2	127.1	195.1	190.4	173.0	236.3	286
287	254.9	130.8	134.9	133 7	127 6	195.8	191.0	173 7	237.1	287
288	255.8	131.3	135.4	134.3	128.1	196 5	191 7	174 3	238 0	288
289	256.7	131.8	135.9	134 8	128 6	197.1	192.4	174 9	238.8	289

TABLE 19*A* (*Continued*)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
290	257.6	132.3	136.4	135.3	129.2	197.8	193.1	175.5	239.6	290
291	258.5	132.7	136.9	135.8	129.7	198.5	193.8	176.2	240.5	291
292	259.4	133.2	137.4	136.3	130.2	199.2	194.4	176.8	241.3	292
293	260.3	133.7	137.9	136.8	130.7	199.9	195.1	177.4	242.1	293
294	261.2	134.2	138.4	137.3	131.2	200.6	195.8	178.1	242.9	294
295	262.0	134.7	138.9	137.8	131.7	201.3	196.5	178.7	243.8	295
296	262.9	135.2	139.4	138.3	132.2	202.0	197.2	179.3	244.6	296
297	263.8	135.7	140.0	138.8	132.7	202.7	197.9	179.9	245.4	297
298	264.7	136.2	140.5	139.4	133.2	203.4	198.6	180.6	246.3	298
299	265.6	136.7	141.0	139.9	133.7	204.1	199.2	181.2	247.1	299
300	266.5	137.2	141.5	140.4	134.2	204.8	199.9	181.8	247.9	300
301	267.4	137.7	142.0	140.9	134.8	205.5	200.6	182.5	248.8	301
302	268.3	138.2	142.5	141.4	135.3	206.2	201.3	183.1	249.6	302
303	269.1	138.7	143.0	141.9	135.8	206.9	202.0	183.7	250.4	303
304	270.0	139.2	143.5	142.4	136.3	207.6	202.7	184.4	251.3	304
305	270.9	139.7	144.0	142.9	136.8	208.3	203.3	185.0	252.1	305
306	271.8	140.2	144.5	143.4	137.3	209.0	204.0	185.6	252.9	306
307	272.7	140.7	145.0	144.0	137.8	209.7	204.7	186.2	253.8	307
308	273.6	141.2	145.5	144.5	138.3	210.4	205.4	186.9	254.6	308
309	274.5	141.7	146.1	145.0	138.8	211.1	206.1	187.5	255.4	309
310	275.4	142.2	146.6	145.5	139.4	211.8	206.8	188.1	256.3	310
311	276.3	142.7	147.1	146.0	139.9	212.5	207.5	188.8	257.1	311
312	277.1	143.2	147.6	146.5	140.4	213.2	208.1	189.4	257.9	312
313	278.0	143.7	148.1	147.0	140.9	213.9	208.8	190.0	258.8	313
314	278.9	144.2	148.6	147.6	141.4	214.6	209.5	190.7	259.6	314
315	279.8	144.7	149.1	148.1	141.9	215.3	210.2	191.3	260.4	315
316	280.7	145.2	149.6	148.6	142.4	216.0	210.9	191.9	261.2	316
317	281.6	145.7	150.1	149.1	143.0	216.6	211.6	192.6	262.1	317
318	282.5	146.2	150.7	149.6	143.5	217.3	212.2	193.2	262.9	318
319	283.4	146.7	151.2	150.1	144.0	218.0	212.9	193.8	263.7	319
320	284.2	147.2	151.7	150.7	144.5	218.7	213.6	194.4	264.6	320
321	285.1	147.7	152.2	151.2	145.0	219.4	214.3	195.1	265.4	321
322	286.0	148.2	152.7	151.7	145.5	220.1	215.5	195.7	266.2	322
323	286.9	148.7	153.2	152.2	146.0	220.8	215.7	196.3	267.1	323
324	287.8	149.2	153.7	152.7	146.6	221.5	216.4	197.0	267.9	324
325	288.7	149.7	154.3	153.2	147.1	222.2	217.0	197.6	268.7	325
326	289.6	150.2	154.8	153.8	147.6	222.9	217.7	198.2	269.6	326
327	290.5	150.7	155.3	154.3	148.1	223.6	218.4	198.9	270.4	327
328	291.4	151.2	155.8	154.8	148.6	224.3	219.1	199.5	271.2	328
329	292.2	151.7	156.3	155.3	149.1	225.0	219.8	200.1	272.1	329

TABLE 19A (*Continued*)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
330	293.1	152.2	156.8	155.8	149.7	225.7	220.5	200.8	272.9	330
331	294.0	152.7	157.3	156.4	150.2	226.4	221.2	201.4	273.7	331
332	294.9	153.2	157.9	156.9	150.7	227.1	221.8	202.0	274.6	332
333	295.8	153.7	158.4	157.4	151.2	227.8	222.5	202.7	275.4	333
334	296.7	154.2	158.9	157.9	151.7	228.5	223.2	203.3	276.2	334
335	297.6	154.7	159.4	158.4	152.3	229.2	223.9	204.0	277.0	335
336	298.5	155.2	159.9	159.0	152.8	229.9	224.6	204.6	277.9	336
337	299.3	155.8	160.5	159.5	153.3	230.6	225.3	205.2	278.7	337
338	300.2	156.3	161.0	160.0	153.8	231.3	226.0	205.9	279.5	338
339	301.1	156.8	161.5	160.5	154.3	232.0	226.7	206.5	280.4	339
340	302.0	157.3	162.0	161.0	154.8	232.7	227.4	207.1	281.2	340
341	302.9	157.8	162.5	161.6	155.4	233.4	228.1	207.8	282.0	341
342	303.8	158.3	163.1	162.1	155.9	234.1	228.7	208.4	282.9	342
343	304.7	158.8	163.6	162.6	156.4	234.8	229.4	209.0	283.7	343
344	305.6	159.3	164.1	163.1	156.9	235.5	230.1	209.7	284.5	344
345	306.5	159.8	164.6	163.7	157.5	236.2	230.8	210.3	285.4	345
346	307.3	160.3	165.1	164.2	158.0	236.9	231.5	211.0	286.2	346
347	308.2	160.8	165.7	164.7	158.5	237.6	232.2	211.6	287.0	347
348	309.1	161.4	166.2	165.2	159.0	238.3	232.9	212.2	287.9	348
349	310.0	161.9	166.7	165.7	159.5	239.0	233.6	212.9	288.7	349
350	310.9	162.4	167.2	166.3	160.1	239.7	234.3	213.5	289.5	350
351	311.8	162.9	167.7	166.8	160.6	240.4	235.0	214.1	290.4	351
352	312.7	163.4	168.3	167.3	161.1	241.1	235.6	214.8	291.2	352
353	313.6	163.9	168.8	167.8	161.6	241.8	236.3	215.4	292.0	353
354	314.4	164.4	169.3	168.4	162.2	242.5	237.0	216.1	292.8	354
355	315.3	164.9	169.8	168.9	162.7	243.2	237.7	216.7	293.7	355
356	316.2	165.4	170.4	169.4	163.2	243.9	238.4	217.3	294.5	356
357	317.1	166.0	170.9	170.0	163.7	244.6	239.1	218.0	295.3	357
358	318.0	166.5	171.4	170.5	164.3	245.3	239.8	218.6	296.2	358
359	318.9	167.0	171.9	171.0	164.8	246.0	240.5	219.2	297.0	359
360	319.8	167.5	172.5	171.5	165.3	246.7	241.2	219.9	297.8	360
361	320.7	168.0	173.0	172.1	165.8	247.4	241.9	220.5	298.7	361
362	321.6	168.5	173.5	172.6	166.4	248.1	242.5	221.2	299.5	362
363	322.4	169.0	174.0	173.1	166.9	248.8	243.2	221.8	300.3	363
364	323.3	169.6	174.6	173.7	167.4	249.5	243.9	222.5	301.2	364
365	324.2	170.1	175.1	174.2	167.9	250.2	244.6	223.1	302.0	365
366	325.1	170.6	175.6	174.7	168.5	250.9	245.3	223.7	302.8	366
367	326.0	171.1	176.1	175.2	169.0	251.6	246.0	224.4	303.6	367
368	326.9	171.6	176.7	175.8	169.5	252.3	246.7	225.0	304.5	368
369	327.8	172.1	177.2	176.3	170.0	253.0	247.4	225.7	305.3	369

TABLE 19A (*Continued*)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
370	328.7	172.7	177.7	176.8	170.6	253.7	248.1	226.3	306.1	370
371	329.5	173.2	178.3	177.4	171.1	254.4	248.8	227.0	307.0	371
372	330.4	173.7	178.8	177.9	171.6	255.1	249.5	227.6	307.8	372
373	331.3	174.2	179.3	178.4	172.2	255.8	250.3	228.3	308.6	373
374	332.2	174.7	179.8	179.0	172.7	256.5	250.9	228.9	309.5	374
375	333.1	175.3	180.4	179.5	173.2	257.2	251.5	229.6	310.3	375
376	334.0	175.8	180.9	180.0	173.7	257.9	252.2	230.2	311.1	376
377	334.9	176.3	181.4	180.6	174.3	258.6	252.9	230.8	312.0	377
378	335.8	176.8	182.0	181.1	174.8	259.3	253.6	231.5	312.8	378
379	336.7	177.3	182.5	181.6	175.3	260.0	254.3	232.1	313.6	379
380	337.5	177.9	183.0	182.1	175.9	260.7	255.0	232.8	314.5	380
381	338.4	178.4	183.6	182.7	176.4	261.4	255.7	233.4	315.3	381
382	339.3	178.9	184.1	183.2	176.9	262.1	256.4	234.1	316.1	382
383	340.2	179.4	184.6	183.6	177.5	262.8	257.1	234.7	316.9	383
384	341.1	180.0	185.2	184.3	178.0	263.5	257.8	235.4	317.8	384
385	342.0	180.5	185.7	184.8	178.5	264.2	258.5	236.0	318.6	385
386	342.9	181.0	186.2	185.4	179.1	264.9	259.2	236.6	319.4	386
387	343.8	181.5	186.8	185.9	179.6	265.6	259.8	237.3	320.3	387
388	344.6	182.0	187.3	186.4	180.1	266.3	260.5	237.9	321.1	388
389	345.5	182.6	187.8	187.0	180.6	267.0	261.2	238.6	321.9	389
390	346.4	183.1	188.4	187.5	181.2	267.7	261.9	239.2	322.8	390
391	347.3	183.6	188.9	188.0	181.7	268.4	262.6	239.9	323.6	391
392	348.2	184.1	189.4	188.6	182.3	269.1	263.3	240.5	324.4	392
393	349.1	184.7	190.0	189.1	182.8	269.8	264.0	241.2	325.2	393
394	350.0	185.2	190.5	189.7	183.3	270.5	264.7	241.8	326.1	394
395	350.9	185.7	191.0	190.2	183.9	271.2	265.4	242.5	326.9	395
396	351.8	186.2	191.6	190.7	184.4	271.9	266.1	243.1	327.7	396
397	352.6	186.8	192.1	191.3	184.9	272.6	266.8	243.8	328.6	397
398	353.5	187.3	192.7	191.8	185.5	273.3	267.5	244.4	329.4	398
399	354.4	187.8	193.2	192.3	186.0	274.0	268.2	245.1	330.2	399
400	355.3	188.4	193.7	192.9	186.5	274.7	268.9	245.7	331.1	400
401	356.2	188.9	194.3	193.4	187.1	275.4	269.6	246.4	331.9	401
402	357.1	189.4	194.8	194.0	187.6	276.1	270.3	247.0	332.7	402
403	358.0	189.9	195.4	194.5	188.1	276.8	271.0	247.7	333.6	403
404	358.9	190.5	195.9	195.0	188.7	277.5	271.7	248.3	334.4	404
405	359.7	191.0	196.4	195.6	189.2	278.2	272.3	249.0	335.2	405
406	360.6	191.5	197.0	196.1	189.8	278.9	273.0	249.6	336.0	406
407	361.5	192.1	197.5	196.7	190.3	279.6	273.7	250.3	336.9	407
408	362.4	192.6	198.1	197.2	190.8	280.3	274.4	251.0	337.7	408
409	363.3	193.1	198.6	197.7	191.4	281.0	275.1	251.6	338.5	409

TABLE 19*A* (*Continued*)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
410	364.2	193.7	199.1	198.3	191.9	281.7	275.8	252.3	339.4	410
411	365.1	194.2	199.7	198.8	192.5	282.4	276.5	252.9	340.2	411
412	366.0	194.7	200.2	199.4	193.0	283.2	277.2	253.6	341.0	412
413	366.9	195.2	200.8	199.9	193.5	283.9	277.9	254.2	341.9	413
414	367.7	195.8	201.3	200.5	194.1	284.6	278.6	254.9	342.7	414
415	368.6	196.3	201.8	201.0	194.6	285.3	279.3	255.5	343.5	415
416	369.5	196.8	202.4	201.6	195.2	286.0	280.0	256.2	344.4	416
417	370.4	197.4	202.9	202.1	195.7	286.7	280.7	256.8	345.2	417
418	371.3	197.9	203.5	202.6	196.2	287.4	281.4	257.5	346.0	418
419	372.2	198.4	204.0	203.2	196.8	288.1	282.1	258.1	346.8	419
420	373.1	199.0	204.6	203.7	197.3	288.8	282.8	258.8	347.7	420
421	374.0	199.5	205.1	204.3	197.9	289.5	283.5	259.4	348.5	421
422	374.8	200.1	205.7	204.8	198.4	290.2	284.2	260.1	349.3	422
423	375.7	200.6	206.2	205.4	198.9	290.9	284.9	260.7	350.2	423
424	376.6	201.1	206.7	205.9	199.5	291.6	285.6	261.4	351.0	424
425	377.5	201.7	207.3	206.5	200.0	292.3	286.3	262.1	351.8	425
426	378.4	202.2	207.8	207.0	200.6	293.0	287.0	262.7	352.7	426
427	379.3	202.8	208.4	207.6	201.1	293.7	287.7	263.4	353.5	427
428	380.2	203.3	208.9	208.1	201.7	294.4	288.4	264.0	354.3	428
429	381.1	203.8	209.5	208.7	202.2	295.1	289.1	264.7	355.1	429
430	382.0	204.4	210.0	209.2	202.7	295.8	289.8	265.4	356.0	430
431	382.8	204.9	210.6	209.8	203.3	296.5	290.5	266.0	356.8	431
432	383.7	205.5	211.1	210.3	203.8	297.2	291.2	266.6	357.6	432
433	384.6	206.0	211.7	210.9	204.4	297.9	291.9	267.3	358.5	433
434	385.5	206.5	212.2	211.4	204.9	298.6	292.6	268.0	359.3	434
435	386.4	207.1	212.8	212.0	205.5	299.3	293.3	268.7	360.1	435
436	387.3	207.6	213.3	212.5	206.0	300.0	294.0	269.3	361.0	436
437	388.2	208.2	213.9	213.1	206.6	300.7	294.7	270.0	361.8	437
438	389.1	208.7	214.4	213.6	207.1	301.4	295.4	270.6	362.6	438
439	390.0	209.2	215.0	214.2	207.7	302.1	296.1	271.3	363.4	439
440	390.8	209.8	215.5	214.7	208.2	302.8	296.8	272.0	364.3	440
441	391.7	210.3	216.1	215.3	208.8	303.5	297.5	272.6	365.1	441
442	392.6	210.9	216.6	215.8	209.3	304.2	298.2	273.3	365.9	442
443	393.5	211.4	217.2	216.4	209.9	304.9	298.9	273.9	366.8	443
444	394.4	212.0	217.8	216.9	210.4	305.6	299.6	274.6	367.6	444
445	395.3	212.5	218.3	217.5	211.0	306.3	300.3	275.3	368.4	445
446	396.2	213.1	218.9	218.0	211.5	307.0	301.0	275.9	369.3	446
447	397.1	213.6	219.4	218.6	212.1	307.7	301 7	276.6	370.1	447
448	397.9	214.1	220.0	219.1	212 6	308.4	302.4	277.2	370.9	448
449	398.8	214.7	220.5	219.7	213.2	309.1	303.1	277.9	371.7	449

TABLE 19A (*Concluded*)

Expressed in milligrams

Cuprous Oxide (Cu_2O)	Copper (Cu)	Glucose	Invert Sugar	Invert Sugar and Sucrose		Lactose	Lactose and Sucrose		Maltose	Cuprous Oxide (Cu_2O)
				0.4 g. Total Sugar	2 g. Total Sugar	$C_{12}H_{22}O_{11}+H_2O$	1 Lactose, 4 Sucrose	1 Lactose, 12 Sucrose	$C_{12}H_{22}O_{11}+H_2O$	
450	399.7	215.2	221.1	220.2	213.7	309.9	303.8	278.6	372.6	450
451	400.6	215.8	221.6	220.8	214.3	310.6	304.5	279.2	373.4	451
452	401.5	216.3	222.2	221.4	214.8	311.3	305.2	279.9	374.2	452
453	402.4	216.9	222.8	221.9	215.4	312.0	305.9	280.5	375.1	453
454	403.3	217.4	223.3	222.5	215.9	312.7	306.6	281.2	375.9	454
455	404.2	218.0	223.9	223.0	216.5	313.4	307.3	281.9	376.7	455
456	405.1	218.5	224.4	223.6	217.0	314.1	308.0	282.5	377.6	456
457	405.9	219.1	225.0	224.1	217.6	314.8	308.7	283.2	378.4	457
458	406.8	219.6	225.5	224.7	218.1	315.5	309.4	283.9	379.2	458
459	407.7	220.2	226.1	225.3	218.7	316.2	310.1	284.5	380.0	459
460	408.6	220.7	226.7	225.8	219.2	316.9	310.8	285.2	380.9	460
461	409.5	221.3	227.3	226.4	219.8	317.6	311.5	285.9	381.7	461
462	410.4	221.8	227.8	226.9	220.3	318.3	312.2	286.5	382.5	462
463	411.3	222.4	228.3	227.5	220.9	319.0	312.9	287.2	383.4	463
464	412.2	222.9	228.9	228.1	221.4	319.7	313.6	287.8	384.2	464
465	413.0	223.5	229.5	228.6	222.0	320.4	314.3	288.5	385.0	465
466	413.9	224.0	230.0	229.2	222.5	321.1	315.0	289.2	385.9	466
467	414.8	224.6	230.6	229.7	223.1	321.8	315.7	289.8	386.7	467
468	415.7	225.1	231.2	230.3	223.7	322.5	316.4	290.5	387.5	468
469	416.6	225.7	231.7	230.9	224.2	323.2	317.0	291.2	388.3	469
470	417.5	226.2	232.3	231.4	224.8	323.9	317.7	291.8	389.2	470
471	418.4	226.8	232.8	232.0	225.3	324.6	318.4	292.5	390.0	471
472	419.3	227.4	233.4	232.5	225.9	325.3	319.1	293.2	390.8	472
473	420.2	227.9	234.0	233.1	226.4	326.0	319.8	293.8	391.7	473
474	421.0	228.3	234.5	233.7	227.0	326.8	320.5	294.5	392.5	474
475	421.9	229.0	235.1	234.2	227.6	327.5	321.2	295.2	393.3	475
476	422.8	229.6	235.7	234.8	228.1	328.2	321.9	295.8	394.2	476
477	423.7	230.1	236.2	235.4	228.7	328.9	322.6	296.5	395.0	477
478	424.6	230.7	236.8	235.9	229.2	329.6	323.3	297.1	395.8	478
479	425.5	231.3	237.4	236.5	229.8	330.3	324.0	297.8	396.6	479
480	426.4	231.8	237.9	237.1	230.3	331.0	324.7	298.5	397.5	480
481	427.3	232.4	238.5	237.6	230.9	331.7	325.4	299.1	398.3	481
482	428.1	232.9	239.1	238.2	231.5	332.4	326.1	299.8	399.1	482
483	429.0	233.5	239.6	238.8	232.0	333.1	326.8	300.5	400.0	483
484	429.9	234.1	240.2	239.3	232.6	333.8	327.5	301.1	400.8	484
485	430.8	234.6	240.8	239.9	233.2	334.5	328.2	301.8	401.6	485
486	431.7	235.2	241.4	240.5	233.7	335.2	328.9	302.5	402.4	486
487	432.6	235.7	241.9	241.0	234.3	335.9	329.6	303.1	403.3	487
488	433.5	236.3	242.5	241.6	234.8	336.6	330.3	303.8	404.1	488
489	434.4	236.9	243.1	242.2	235.4	337.3	331.0	304.5	404.9	489
490	435.3	237.4	243.6	242.7	236.0	338.0	331.7	305.1	405.8	490

TABLE 19*B**

HAMMOND'S REVISED MUNSON AND WALKER TABLE FOR DETERMINING GLUCOSE, FRUCTOSE, INVERT SUGAR ALONE, AND INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.3, 0.4, OR 2 g. TOTAL SUGARS)†

Copper	Cuprous Oxide	Glucose	Invert Sugar	Invert Sugar and Sucrose			Fructose	Copper
				0.3 g. of Total Sugar	0.4 g. of Total Sugar	2.0 g. of Total Sugar		
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
10	11.3	4.6	5.2	3.2	2.9	.....	5.1	10
11	12.4	5.1	5.7	3.7	3.4	.....	5.6	11
12	13.5	5.6	6.2	4.2	3.9	.....	6.1	12
13	14.6	6.0	6.7	4.8	4.4	.....	6.7	13
14	15.8	6.5	7.2	5.3	4.9	.....	7.2	14
15	16.9	7.0	7.7	5.8	5.4	.....	7.7	15
16	18.0	7.5	8.2	6.3	5.9	.....	8.3	16
17	19.1	8.0	8.7	6.8	6.4	.....	8.8	17
18	20.3	8.5	9.2	7.3	6.9	.....	9.3	18
19	21.4	8.9	9.7	7.8	7.4	.....	9.9	19
20	22.5	9.4	10.2	8.3	7.9	1.9	10.4	20
21	23.6	9.9	10.7	8.8	8.4	2.4	10.9	21
22	24.8	10.4	11.2	9.3	8.9	2.9	11.5	22
23	25.9	10.9	11.7	9.9	9.5	3.4	12.0	23
24	27.0	11.4	12.3	10.4	10.0	3.9	12.5	24
25	28.1	11.9	12.8	10.9	10.5	4.4	13.1	25
26	29.3	12.3	13.3	11.4	11.0	4.9	13.6	26
27	30.4	12.8	13.8	11.9	11.5	5.5	14.2	27
28	31.5	13.3	14.3	12.4	12.0	6.0	14.7	28
29	32.6	13.8	14.8	12.9	12.5	6.5	15.2	29
30	33.8	14.3	15.3	13.4	13.0	7.0	15.8	30
31	34.9	14.8	15.8	14.0	13.5	7.5	16.3	31
32	36.0	15.3	16.3	14.5	14.1	8.0	16.8	32
33	37.2	15.7	16.8	15.0	14.6	8.5	17.4	33
34	38.3	16.2	17.3	15.5	15.1	9.0	17.9	34
35	39.4	16.7	17.8	16.0	15.6	9.5	18.4	35
36	40.5	17.2	18.3	16.5	16.1	10.1	19.0	36
37	41.7	17.7	18.9	17.0	16.6	10.6	19.5	37
38	42.8	18.2	19.4	17.6	17.1	11.1	20.1	38
39	43.9	18.7	19.9	18.1	17.6	11.6	20.6	39
40	45.0	19.2	20.4	18.6	18.2	12.1	21.1	40
41	46.2	19.7	20.9	19.1	18.7	12.6	21.7	41
42	47.3	20.1	21.4	19.6	19.2	13.1	22.2	42
43	48.4	20.6	21.9	20.1	19.7	13.7	22.8	43
44	49.5	21.1	22.4	20.7	20.2	14.2	23.3	44
45	50.7	21.6	22.9	21.2	20.7	14.7	23.8	45
46	51.8	22.1	23.5	21.7	21.3	15.2	24.4	46
47	52.9	22.6	24.0	22.2	21.8	15.7	24.9	47
48	54.0	23.1	24.5	22.7	22.3	16.2	25.4	48
49	55.2	23.6	25.0	23.2	22.8	16.8	26.0	49

* See text, pp. 801 and 813. Taken from *J. Research Nat. Bur. Standards*, **24**, 589–596 (1940).

† The values in the table for concentrations of reducing sugar less than 20 mg. are extrapolated and should be used with caution and only for approximate determinations.

TABLE 19*B* (*Continued*)

Copper	Cuprous Oxide	Glucose	Invert Sugar	Invert Sugar and Sucrose			Fructose	Copper
				0.3 g. of Total Sugar	0.4 g. of Total Sugar	2.0 g. of Total Sugar		
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
50	56.3	24.1	25.5	23.8	23.3	17.3	26.5	50
51	57.4	24.6	26.0	24.3	23.8	17.8	27.1	51
52	58.5	25.1	26.5	24.8	24.3	18.3	27.6	52
53	59.7	25.6	27.0	25.3	24.9	18.8	28.2	53
54	60.8	26.1	27.6	25.8	25.4	19.3	28.7	54
55	61.9	26.5	28.1	26.3	25.9	19.9	29.2	55
56	63.0	27.0	28.6	26.9	26.4	20.4	29.8	56
57	64.2	27.5	29.1	27.4	26.9	20.9	30.3	57
58	65.3	28.0	29.6	27.9	27.5	21.4	30.9	58
59	66.4	28.5	30.1	28.4	28.0	21.9	31.4	59
60	67.6	29.0	30.6	28.9	28.5	22.5	31.9	60
61	68.7	29.5	31.2	29.5	29.0	23.0	32.5	61
62	69.8	30.0	31.7	30.0	29.5	23.5	33.0	62
63	70.9	30.5	32.2	30.5	30.1	24.0	33.6	63
64	72.1	31.0	32.7	31.0	30.6	24.5	34.1	64
65	73.2	31.5	33.2	31.6	31.1	25.1	34.7	65
66	74.3	32.0	33.7	32.1	31.6	25.6	35.2	66
67	75.4	32.5	34.3	32.6	32.1	26.1	35.8	67
68	76.6	33.0	34.8	33.1	32.7	26.6	36.3	68
69	77.7	33.5	35.3	33.6	33.2	27.1	36.8	69
70	78.8	34.0	35.8	34.2	33.7	27.7	37.4	70
71	79.9	34.5	36.3	34.7	34.2	28.2	37.9	71
72	81.1	35.0	36.8	35.2	34.7	28.7	38.5	72
73	82.2	35.5	37.4	35.7	35.3	29.2	39.0	73
74	83.3	36.0	37.9	36.3	35.8	29.8	39.6	74
75	84.4	36.5	38.4	36.8	36.3	30.3	40.1	75
76	85.6	37.0	38.9	37.3	36.8	30.8	40.7	76
77	86.7	37.5	39.4	37.8	37.4	31.3	41.2	77
78	87.8	38.0	40.0	38.4	37.9	31.9	41.7	78
79	88.9	38.5	40.5	38.9	38.4	32.4	42.3	79
80	90.1	39.0	41.0	39.4	38.9	32.9	42.8	80
81	91.2	39.5	41.5	39.9	39.5	33.4	43.4	81
82	92.3	40.0	42.0	40.5	40.0	34.0	43.9	82
83	93.4	40.5	42.6	41.0	40.5	34.5	44.5	83
84	94.6	41.0	43.1	41.5	41.0	35.0	45.0	84
85	95.7	41.5	43.6	42.0	41.6	35.5	45.6	85
86	96.8	42.0	44.1	42.6	42.1	36.1	46.1	86
87	97.9	42.5	44.7	43.1	42.6	36.6	46.7	87
88	99.1	43.0	45.2	43.6	43.1	37.1	47.2	88
89	100.2	43.5	45.7	44.1	43.7	37.6	47.8	89
90	101.3	44.0	46.2	44.7	44.2	38.2	48.3	90
91	102.5	44.5	46.7	45.2	44.7	38.7	48.9	91
92	103.6	45.0	47.3	45.7	45.2	39.2	49.4	92
93	104.7	45.5	47.8	46.3	45.8	39.8	50.0	93
94	105.8	46.0	48.3	46.8	46.3	40.3	50.5	94

TABLE 19*B* (*Continued*)

Copper	Cuprous Oxide	Glucose	Invert Sugar	Invert Sugar and Sucrose			Fructose	Copper
				0.3 g. of Total Sugar	0.4 g. of Total Sugar	2.0 g. of Total Sugar		
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
95	107.0	46.5	48.8	47.3	46.8	40.8	51.1	95
96	108.1	47.0	49.4	47.8	47.4	41.3	51.6	96
97	109.2	47.5	49.9	48.4	47.9	41.9	52.2	97
98	110.3	48.0	50.4	48.9	48.4	42.4	52.7	98
99	111.5	48.5	50.9	49.4	48.9	42.9	53.3	99
100	112.6	49.0	51.5	50.0	49.5	43.5	53.8	100
101	113.7	49.5	52.0	50.5	50.0	44.0	54.4	101
102	114.8	50.0	52.5	51.0	50.5	44.5	54.9	102
103	116.0	50.6	53.0	51.6	51.1	45.1	55.5	103
104	117.1	51.1	53.6	52.1	51.6	45.6	56.0	104
105	118.2	51.6	54.1	52.6	52.1	46.1	56.6	105
106	119.3	52.1	54.6	53.1	52.7	46.7	57.1	106
107	120.5	52.6	55.2	53.7	53.2	47.2	57.7	107
108	121.6	53.1	55.7	54.2	53.7	47.7	58.2	108
109	122.7	53.6	56.2	54.7	54.2	48.3	58.8	109
110	123.8	54.1	56.7	55.3	54.8	48.8	59.3	110
111	125.0	54.6	57.3	55.8	55.3	49.3	59.9	111
112	126.1	55.1	57.8	56.3	55.8	49.9	60.4	112
113	127.2	55.6	58.3	56.9	56.4	50.4	61.0	113
114	128.3	56.1	58.9	57.4	56.9	50.9	61.6	114
115	129.5	56.7	59.4	57.9	57.4	51.5	62.1	115
116	130.6	57.2	59.9	58.5	58.0	52.0	62.7	116
117	131.7	57.7	60.4	59.0	58.5	52.5	63.2	117
118	132.8	58.2	61.0	59.5	59.0	53.1	63.8	118
119	134.0	58.7	61.5	60.1	59.6	53.6	64.3	119
120	135.1	59.2	62.0	60.6	60.1	54.1	64.9	120
121	136.2	59.7	62.6	61.2	60.7	54.7	65.4	121
122	137.4	60.2	63.1	61.7	61.2	55.2	66.0	122
123	138.5	60.7	63.6	62.2	61.7	55.8	66.5	123
124	139.6	61.3	64.2	62.8	62.3	56.3	67.1	124
125	140.7	61.8	64.7	63.3	62.8	56.8	67.7	125
126	141.9	62.3	65.2	63.8	63.3	57.4	68.2	126
127	143.0	62.8	65.8	64.4	63.9	57.9	68.8	127
128	144.1	63.3	66.3	64.9	64.4	58.4	69.3	128
129	145.2	63.8	66.8	65.4	64.9	59.0	69.9	129
130	146.4	64.3	67.4	66.0	65.5	59.5	70.4	130
131	147.5	64.9	67.9	66.5	66.0	60.1	71.0	131
132	148.6	65.4	68.4	67.1	66.6	60.6	71.6	132
133	149.7	65.9	69.0	67.6	67.1	61.1	72.1	133
134	150.9	66.4	69.5	68.1	67.6	61.7	72.7	134
135	152.0	66.9	70.0	68.7	68.2	62.2	73.2	135
136	153.1	67.4	70.6	69.2	68.7	62.8	73.8	136
137	154.2	68.0	71.1	69 8	69.3	63.3	74.3	137
138	155.4	68.5	71.6	70.3	69.8	63.9	74.9	138
139	156.5	69.0	72.2	70 8	70.3	64.4	75.5	139

TABLE 19*B* (*Continued*)

Copper	Cuprous Oxide	Glucose	Invert Sugar	Invert Sugar and Sucrose			Fructose	Copper
				0.3 g. of Total Sugar	0.4 g. of Total Sugar	2.0 g. of Total Sugar		
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
140	157.6	69.5	72.7	71.4	70.9	64.9	76.0	140
141	158.7	70.0	73.2	71.9	71.4	65.5	76.6	141
142	159.9	70.5	73.8	72.5	72.0	66.0	77.1	142
143	161.0	71.1	74.3	73.0	72.5	66.6	77.7	143
144	162.1	71.6	74.9	73.5	73.0	67.1	78.3	144
145	163.2	72.1	75.4	74.1	73.6	67.7	78.8	145
146	164.4	72.6	75.9	74.6	74.1	68.2	79.4	146
147	165.5	73.1	76.5	75.2	74.7	68.7	80.0	147
148	166.6	73.7	77.0	75.7	75.2	69.3	80.5	148
149	167.8	74.2	77.6	76.3	75.7	69.8	81.1	149
150	168.9	74.7	78.1	76.8	76.3	70.4	81.6	150
151	170.0	75.2	78.6	77.3	76.8	70.9	82.2	151
152	171.1	75.7	79.2	77.9	77.4	71.5	82.8	152
153	172.3	76.3	79.7	78.4	77.9	72.0	83.3	153
154	173.4	76.8	80.3	79.0	78.5	72.6	83.9	154
155	174.5	77.3	80.8	79.5	79.0	73.1	84.4	155
156	175.6	77.8	81.3	80.1	79.6	73.7	85.0	156
157	176.8	78.3	81.9	80.6	80.1	74.2	85.6	157
158	177.9	78.9	82.4	81.2	80.6	74.8	86.1	158
159	179.0	79.4	83.0	81.7	81.2	75.3	86.7	159
160	180.1	79.9	83.5	82.2	81.7	75.9	87.3	160
161	181.3	80.4	84.0	82.8	82.3	76.4	87.8	161
162	182.4	81.0	84.6	83.3	82.8	77.0	88.4	162
163	183.5	81.5	85.1	83.9	83.4	77.5	89.0	163
164	184.6	82.0	85.7	84.4	83.9	78.1	89.5	164
165	185.8	82.5	86.2	85.0	84.5	78.6	90.1	165
166	186.9	83.1	86.8	85.5	85.0	79.2	90.6	166
167	188.0	83.6	87.3	86.1	85.6	79.7	91.2	167
168	189.1	84.1	87.8	86.6	86.1	80.3	91.8	168
169	190.3	84.6	88.4	87.2	86.7	80.8	92.3	169
170	191.4	85.2	88.9	87.7	87.2	81.4	92.9	170
171	192.5	85.7	89.5	88.3	87.8	81.9	93.5	171
172	193.6	86.2	90.0	88.8	88.3	82.5	94.0	172
173	194.8	86.7	90.6	89.4	88.9	83.0	94.6	173
174	195.9	87.3	91.1	89.9	89.4	83.6	95.2	174
175	197.0	87.8	91.7	90.5	90.0	84.1	95.7	175
176	198.1	88.3	92.2	91.0	90.5	84.7	96.3	176
177	199.3	88.9	92.8	91.6	91.1	85.2	96.9	177
178	200.4	89.4	93.3	92.1	91.6	85.8	97.4	178
179	201.5	89.9	93.8	92.7	92.2	86.3	98.0	179
180	202.7	90.4	94.4	93.2	92.7	86.9	98.6	180
181	203.8	91.0	94.9	93.8	93.3	87.4	99.2	181
182	204.9	91.5	95.5	94.3	93.8	88.0	99.7	182
183	206.0	92.0	96.0	94.9	94.4	88.6	100.3	183
184	207.2	92.6	96.6	95.4	94.9	89.1	100.9	184

TABLE 19*B* (*Continued*)

Copper	Cuprous Oxide	Glucose	Invert Sugar	Invert Sugar and Sucrose			Fructose	Copper
				0.3 g. of Total Sugar	0.4 g. of Total Sugar	2.0 g. of Total Sugar		
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
185	208.3	93.1	97.1	96.0	95.5	89.7	101.4	185
186	209.4	93.6	97.7	96.5	96.0	90.2	102.0	186
187	210.5	94.2	98.2	97.1	96.6	90.8	102.6	187
188	211.7	94.7	98.8	97.6	97.1	91.3	103.1	188
189	212.8	95.2	99.3	98.2	97.7	91.9	103.7	189
190	213.9	95.7	99.9	98.7	98.2	92.4	104.3	190
191	215.0	96.3	100.4	99.3	98.8	93.0	104.8	191
192	216.2	96.8	101.0	99.9	99.4	93.6	105.4	192
193	217.3	97.3	101.5	100.4	99.9	94.1	106.0	193
194	218.4	97.9	102.1	101.0	100.5	94.7	106.6	194
195	219.5	98.4	102.6	101.5	101.0	95.2	107.1	195
196	220.7	98.9	103.2	102.1	101.6	95.8	107.7	196
197	221.8	99.5	103.7	102.6	102.1	96.4	108.3	197
198	222.9	100.0	104.3	103.2	102.7	96.9	108.8	198
199	224.0	100.5	104.8	103.7	103.2	97.5	109.4	199
200	225.2	101.1	105.4	104.3	103.8	98.0	110.0	200
201	226.3	101.6	106.0	104.9	104.4	98.6	110.6	201
202	227.4	102.2	106.5	105.4	104.9	99.2	111.1	202
203	228.5	102.7	107.1	106.0	105.5	99.7	111.7	203
204	229.7	103.2	107.6	106.5	106.0	100.3	112.3	204
205	230.8	103.8	108.2	107.1	106.6	100.9	112.9	205
206	231.9	104.3	108.7	107.6	107.2	101.4	113.4	206
207	233.1	104.8	109.3	108.2	107.7	102.0	114.0	207
208	234.2	105.4	109.8	108.8	108.3	102.5	114.6	208
209	235.3	105.9	110.4	109.3	108.8	103.1	115.2	209
210	236.4	106.5	110.9	109.9	109.4	103.7	115.7	210
211	237.6	107.0	111.5	110.4	110.0	104.2	116.3	211
212	238.7	107.5	112.1	111.0	110.5	104.8	116.9	212
213	239.8	108.1	112.6	111.6	111.1	105.4	117.5	213
214	240.9	108.6	113.2	112.1	111.6	105.9	118.0	214
215	242.1	109.2	113.7	112.7	112.2	106.5	118.6	215
216	243.1	109.7	114.3	113.2	112.8	107.1	119.2	216
217	244.3	110.2	114.9	113.8	113.3	107.6	119.8	217
218	245.4	110.8	115.4	114.4	113.9	108.2	120.3	218
219	246.6	111.3	116.0	114.9	114.4	108.8	120.9	219
220	247.7	111.9	116.5	115.5	115.0	109.3	121.5	220
221	248.8	112.4	117.1	116.1	115.6	109.9	122.1	221
222	249.9	112.9	117.6	116.6	116.1	110.5	122.6	222
223	251.1	113.5	118.2	117.2	116.7	111.0	123.2	223
224	252.2	114.0	118.8	117.7	117.3	111.6	123.8	224
225	253.3	114.6	119.3	118.3	117.8	112.2	124.4	225
226	254.4	115.1	119.9	118.9	118.4	112.7	125.0	226
227	255.6	115.7	120.4	119.4	119.0	113.3	125.5	227
228	256.7	116.2	121.0	120.0	119.5	113.9	126.1	228
229	257.8	116.7	121.6	120.6	120.1	114.4	126.7	229

TABLE 19*B* (*Continued*)

Copper	Cuprous Oxide	Glucose	Invert Sugar	Invert Sugar and Sucrose			Fructose	Copper
				0.3 g. of Total Sugar	0.4 g. of Total Sugar	2.0 g. of Total Sugar		
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
230	258.9	117.3	122.1	121.1	120.7	115.0	127.3	230
231	260.1	117.8	122.7	121.7	121.2	115.6	127.9	231
232	261.2	118.4	123.3	122.3	121.8	116.2	128.4	232
233	262.3	118.9	123.8	122.8	122.4	116.7	129.0	233
234	263.4	119.5	124.4	123.4	122.9	117.3	129.6	234
235	264.6	120.0	124.9	124.0	123.5	117.9	130.2	235
236	265.7	120.6	125.5	124.5	124.1	118.4	130.8	236
237	266.8	121.1	126.1	125.1	124.6	119.0	131.3	237
238	268.0	121.7	126.6	125.7	125.2	119.6	131.9	238
239	269.1	122.2	127.2	126.2	125.8	120.2	132.5	239
240	270.2	122.7	127.8	126.8	126.3	120.7	133.1	240
241	271.3	123.3	128.3	127.4	126.9	121.3	133.7	241
242	272.5	123.8	128.9	127.9	127.5	121.9	134.2	242
243	273.6	124.4	129.5	128.5	128.0	122.5	134.8	243
244	274.7	124.9	130.0	129.1	128.6	123.0	135.4	244
245	275.8	125.5	130.6	129.6	129.2	123.6	136.0	245
246	277.0	126.0	131.2	130.2	129.8	124.2	136.6	246
247	278.1	126.6	131.7	130.8	130.3	124.8	137.2	247
248	279.2	127.1	132.3	131.3	130.9	125.3	137.7	248
249	280.3	127.7	132.9	131.9	131.5	125.9	138.3	249
250	281.5	128.2	133.4	132.5	132.0	126.5	138.9	250
251	282.6	128.8	134.0	133.1	132.6	127.1	139.5	251
252	283.7	129.3	134.6	133.6	133.2	127.6	140.1	252
253	284.8	129.9	135.1	134.2	133.8	128.2	140.7	253
254	286.0	130.4	135.7	134.8	134.3	128.8	141.3	254
255	287.1	131.0	136.3	135.3	134.9	129.4	141.8	255
256	288.2	131.6	136.8	135.9	135.5	130.0	142.4	256
257	289.3	132.1	137.4	136.5	136.0	130.5	143.0	257
258	290.5	132.7	138.0	137.1	136.6	131.1	143.6	258
259	291.6	133.2	138.6	137.6	137.2	131.7	144.2	259
260	292.7	133.8	139.1	138.2	137.8	132.3	144.8	260
261	293.8	134.3	139.7	138.8	138.3	132.9	145.4	261
262	295.0	134.9	140.3	139.4	138.9	133.4	145.9	262
263	296.1	135.4	140.8	139.9	139.5	134.0	146.5	263
264	297.2	136.0	141.4	140.5	140.1	134.6	147.1	264
265	298.3	136.5	142.0	141.1	140.7	135.2	147.7	265
266	299.5	137.1	142.6	141.7	141.2	135.8	148.3	266
267	300.6	137.7	143.1	142.2	141.8	136.3	148.9	267
268	301.7	138.2	143.7	142.8	142.4	136.9	149.5	268
269	302.9	138.8	144.3	143.4	143.0	137.5	150.1	269
270	304.0	139.3	144.8	144.0	143.5	138.1	150.6	270
271	305.1	139.9	145.4	144.5	144.1	138.7	151.2	271
272	306.2	140.4	146.0	145.1	144.7	139.3	151.8	272
273	307.4	141.0	146.6	145.7	145.3	139.8	152.4	273
274	308.5	141.6	147.1	146.3	145.9	140.4	153.0	274

TABLE 19*B* (*Continued*)

Copper	Cuprous Oxide	Glucose	Invert Sugar	Invert Sugar and Sucrose			Fructose	Copper
				0.3 g. of Total Sugar	0.4 g. of Total Sugar	2.0 g. of Total Sugar		
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
275	309.6	142.1	147.7	146.8	146.4	141.0	153.6	275
276	310.7	142.7	148.3	147.4	147.0	141.6	154.2	276
277	311.9	143.2	148.9	148.0	147.6	142.2	154.8	277
278	313.0	143.8	149.4	148.6	148.2	142.8	155.4	278
279	314.1	144.4	150.0	149.2	148.8	143.4	156.0	279
280	315.2	144.9	150.6	149.7	149.3	143.9	156.5	280
281	316.4	145.5	151.2	150.3	149.9	144.5	157.1	281
282	317.5	146.0	151.8	150.9	150.5	145.1	157.7	282
283	318.6	146.6	152.3	151.5	151.1	145.7	158.3	283
284	319.7	147.2	152.9	152.1	151.7	146.3	158.9	284
285	320.9	147.7	153.5	152.6	152.2	146.9	159.5	285
286	322.0	148.3	154.1	153.2	152.8	147.5	160.1	286
287	323.1	148.8	154.6	153.8	153.4	148.1	160.7	287
288	324.2	149.4	155.2	154.4	154.0	148.6	161.3	288
289	325.4	150.0	155.8	155.0	154.6	149.2	161.9	289
290	326.5	150.5	156.4	155.5	155.2	149.8	162.5	290
291	327.6	151.1	157.0	156.1	155.7	150.4	163.1	291
292	328.7	151.7	157.5	156.7	156.3	151.0	163.7	292
293	329.9	152.2	158.1	157.3	156.9	151.6	164.3	293
294	331.0	152.8	158.7	157.9	157.5	152.2	164.9	294
295	332.1	153.4	159.3	158.5	158.1	152.8	165.4	295
296	333.3	153.9	159.9	159.0	158.7	153.4	166.0	296
297	334.4	154.5	160.5	159.6	159.3	154.0	166.6	297
298	335.5	155.1	161.0	160.2	159.9	154.6	167.2	298
299	336.6	155.6	161.6	160.8	160.4	155.2	167.8	299
300	337.8	156.2	162.2	161.4	161.0	155.7	168.4	300
301	338.9	156.8	162.8	162.0	161.6	156.3	169.0	301
302	340.0	157.3	163.4	162.5	162.2	156.9	169.6	302
303	341.1	157.9	164.0	163.1	162.8	157.5	170.2	303
304	342.3	158.5	164.5	163.7	163.4	158.1	170.8	304
305	343.4	159.0	165.1	164.3	164.0	158.7	171.4	305
306	344.5	159.6	165.7	164.9	164.6	159.3	172.0	306
307	345.6	160.2	166.3	165.5	165.1	159.9	172.6	307
308	346.8	160.7	166.9	166.1	165.7	160.5	173.2	308
309	347.9	161.3	167.5	166.7	166.3	161.1	173.8	309
310	349.0	161.9	168.0	167.2	166.9	161.7	174.4	310
311	350.1	162.5	168.6	167.8	167.5	162.3	175.0	311
312	351.3	163.0	169.2	168.4	168.1	162.9	175.6	312
313	352.4	163.6	169.8	169.0	168.7	163.5	176.2	313
314	353.5	164.2	170.4	169.6	169.3	164.1	176.8	314
315	354.6	164.7	171.0	170.2	169.9	164.7	177.4	315
316	355.8	165.3	171.6	170.8	170.5	165.3	178.0	316
317	356.9	165.9	172.2	171.4	171.1	165.9	178.6	317
318	358.0	166.5	172.8	172.0	171.7	166.5	179.2	318
319	359.1	167.0	173.3	172.6	172.2	167.1	179.8	319

TABLE 19*B* (*Continued*)

Copper	Cuprous Oxide	Glucose	Invert Sugar	Invert Sugar and Sucrose			Fructose	Copper
				0.3 g. of Total Sugar	0.4 g. of Total Sugar	2.0 g. of Total Sugar		
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
320	360.3	167.6	173.9	173.1	172.8	167.7	180.4	320
321	361.4	168.2	174.5	173.7	173.4	168.3	181.0	321
322	362.5	168.8	175.1	174.3	174.0	168.9	181.6	322
323	363.6	169.3	175.7	174.9	174.6	169.5	182.2	323
324	364.8	169.9	176.3	175.5	175.2	170.1	182.8	324
325	365.9	170.5	176.9	176.1	175.8	170.7	183.4	325
326	367.0	171.1	177.5	176.7	176.4	171.3	184.0	326
327	368.2	171.6	178.1	177.3	177.0	171.9	184.6	327
328	369.3	172.2	178.7	177.9	177.6	172.5	185.2	328
329	370.4	172.8	179.2	178.5	178.2	173.1	185.8	329
330	371.5	173.4	179.8	179.1	178.8	173.7	186.4	330
331	372.7	173.9	180.4	179.7	179.4	174.3	187.0	331
332	373.8	174.5	181.0	180.3	180.0	174.9	187.6	332
333	374.9	175.1	181.6	180.9	180.6	175.5	188.2	333
334	376.0	175.7	182.2	181.5	181.2	176.1	188.8	334
335	377.2	176.3	182.8	182.1	181.8	176.7	189.4	335
336	378.3	176.8	183.4	182.6	182.4	177.3	190.1	336
337	379.4	177.4	184.0	183.2	183.0	178.0	190.7	337
338	380.5	178.0	184.6	183.8	183.6	178.6	191.3	338
339	381.7	178.6	185.2	184.4	184.2	179.2	191.9	339
340	382.8	179.2	185.8	185.0	184.8	179.8	192.5	340
341	383.9	179.7	186.4	185.6	185.4	180.4	193.1	341
342	385.0	180.3	187.0	186.2	186.0	181.0	193.7	342
343	386.2	180.9	187.6	186.8	186.6	181.6	194.3	343
344	387.3	181.5	188.2	187.4	187.2	182.2	194.9	344
345	388.4	182.1	188.8	188.0	187.8	182.8	195.5	345
346	389.5	182.7	189.4	188.6	188.4	183.4	196.1	346
347	390.7	183.2	190.0	189.2	189.0	184.0	196.7	347
348	391.8	183.8	190.6	189.8	189.6	184.6	197.3	348
349	392.9	184.4	191.2	190.4	190.2	185.3	197.9	349
350	394.0	185.0	191.8	191.0	190.8	185.9	198.5	350
351	395.2	185.6	192.4	191.6	191.4	186.5	199.2	351
352	396.3	186.2	193.0	192.2	192.0	187.1	199.8	352
353	397.4	186.8	193.6	192.8	192.6	187.7	200.4	353
354	398.5	187.3	194.2	193.4	193.2	188.3	201.0	354
355	399.7	187.9	194.8	194.0	193.8	188.9	201.6	355
356	400.8	188.5	195.4	194.6	194.4	189.5	202.2	356
357	401.9	189.1	196.0	195.2	195.0	190.2	202.8	357
358	403.1	189.7	196.6	195.8	195.7	190.8	203.4	358
359	404.2	190.3	197.2	196.4	196.3	191.4	204.0	359
360	405.3	190.9	197.8	197.1	196.9	192.0	204.7	360
361	406.4	191.5	198.4	197.7	197.5	192.6	205.3	361
362	407.6	192.0	199.0	198.3	198.1	193.2	205.9	362
363	408.7	192.6	199.6	198.9	198.7	193.9	206.5	363
364	409.8	193.2	200.2	199.5	199.3	194.5	207.1	364

TABLE 19*B* (*Continued*)

Copper	Cuprous Oxide	Glucose	Invert Sugar	Invert Sugar and Sucrose			Fructose	Copper
				0.3 g. of Total Sugar	0.4 g. of Total Sugar	2.0 g. of Total Sugar		
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
365	410.9	193.8	200.8	200.1	199.9	195.1	207.7	365
366	412.1	194.4	201.4	200.7	200.5	195.7	208.3	366
367	413.2	195.0	202.0	201.3	201.1	196.3	209.0	367
368	414.3	195.6	202.6	201.9	201.7	196.9	209.6	368
369	415.4	196.2	203.2	202.5	202.4	197.6	210.2	369
370	416.6	196.8	203.8	203.1	203.0	198.2	210.8	370
371	417.7	197.4	204.4	203.7	203.6	198.8	211.4	371
372	418.8	198.0	205.0	204.3	204.2	199.4	212.0	372
373	419.9	198.5	205.7	204.9	204.8	200.0	212.6	373
374	421.1	199.1	206.3	205.6	205.4	200.7	213.3	374
375	422.2	199.7	206.9	206.2	206.0	201.3	213.9	375
376	423.3	200.3	207.5	206.8	206.6	201.9	214.5	376
377	424.4	200.9	208.1	207.4	207.3	202.5	215.1	377
378	425.6	201.5	208.7	208.0	207.9	203.1	215.7	378
379	426.7	202.1	209.3	208.6	208.5	203.8	216.3	379
380	427.8	202.7	209.9	209.2	209.1	204.4	217.0	380
381	428.9	203.3	210.5	209.8	209.7	205.0	217.6	381
382	430.1	203.9	211.1	210.4	210.3	205.6	218.2	382
383	431.2	204.5	211.8	211.1	211.0	206.3	218.8	383
384	432.3	205.1	212.4	211.7	211.6	206.9	219.5	384
385	433.5	205.7	213.0	212.3	212.2	207.5	220.1	385
386	434.6	206.3	213.6	212.9	212.8	208.1	220.7	386
387	435.7	206.9	214.2	213.5	213.4	208.8	221.3	387
388	436.8	207.5	214.8	214.1	214.0	209.4	221.9	388
389	438.0	208.1	215.4	214.7	214.7	210.0	222.6	389
390	439.1	208.7	216.0	215.4	215.3	210.6	223.2	390
391	440.2	209.3	216.7	216.0	215.9	211.3	223.8	391
392	441.3	209.9	217.3	216.6	216.5	211.9	224.4	392
393	442.5	210.5	217.9	217.2	217.1	212.5	225.1	393
394	443.6	211.1	218.5	217.8	217.8	213.2	225.7	394
395	444.7	211.7	219.1	218.5	218.4	213.8	226.3	395
396	445.8	212.3	219.8	219.1	219.0	214.4	226.9	396
397	447.0	212.9	220.4	219.7	219.6	215.1	227.6	397
398	448.1	213.5	221.0	220.3	220.3	215.7	228.2	398
399	449.2	214.1	221.6	220.9	220.9	216.3	228.8	399
400	450.3	214.7	222.2	221.5	221.5	217.0	229.4	400
401	451.5	215.3	222.9	222.2	222.1	217.6	230.1	401
402	452.6	215.9	223.5	222.8	222.8	218.2	230.7	402
403	453.7	216.5	224.1	223.4	223.4	218.9	231.3	403
404	454.8	217.1	224.7	224.0	224.0	219.5	232.0	404
405	456.0	217.8	225.4	224.7	224.7	220.1	232.6	405
406	457.1	218.4	226.0	225.3	225.3	220.8	233.2	406
407	458.2	219.0	226.6	225.9	225.9	221.4	233.9	407
408	459.3	219.6	227.2	226.6	226.5	222.0	234.5	408
409	460.5	220.2	227.9	227.2	227.2	222.7	235.1	409

TABLE 19*B* (*Concluded*)

Copper	Cuprous Oxide	Glucose	Invert Sugar	Invert Sugar and Sucrose			Fructose	Copper
				0.3 g. of Total Sugar	0.4 g. of Total Sugar	2.0 g. of Total Sugar		
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
410	461.6	220.8	228.5	227.8	227.8	223.3	235.8	410
411	462.7	221.4	229.1	228.4	228.4	224.0	236.4	411
412	463.8	222.0	229.7	229.1	229.1	224.6	237.1	412
413	465.0	222.6	230.4	229.7	229.7	225.3	237.7	413
414	466.1	223.3	231.0	230.4	230.4	225.9	238.4	414
415	467.2	223.9	231.7	231.0	231.0	226.6	239.0	415
416	468.4	224.5	232.3	231.6	231.7	227.2	239.7	416
417	469.5	225.1	232.9	232.3	232.3	227.8	240.3	417
418	470.6	225.7	233.6	232.9	232.9	228.5	241.0	418
419	471.7	226.3	234.2	233.5	233.6	229.1	241.6	419
420	472.9	227.0	234.8	234.2	234.2	229.8	242.2	420
421	474.0	227.6	235.5	234.8	234.9	230.4	242.9	421
422	475.1	228.2	236.1	235.5	235.5	231.1	243.6	422
423	476.2	228.8	236.8	236.2	236.2	231.8	244.3	423
424	477.4	229.5	237.5	236.8	236.9	232.4	244.9	424
425	478.5	230.1	238.1	237.5	237.5	233.1	245.6	425
426	479.6	230.7	238.8	238.2	238.2	233.8	246.3	426
427	480.7	231.4	239.5	238.8	238.9	234.5	247.0	427
428	481.9	232.0	240.2	239.5	239.6	235.1	247.8	428
429	483.0	232.7	240.8	240.2	240.3	235.8	248.5	429
430	484.1	233.3	241.5	240.9	241.0	236.5	249.2	430
431	485.2	234.0	242.3	241.7	241.7	237.2	250.0	431
432	486.4	234.7	243.0	242.4	242.5	238.0	250.8	432
433	487.5	235.3	243.8	243.2	243.3	238.7	251.6	433
434	488.6	236.1	244.7	244.1	244.2	239.6	252.7	434
435	489.7	236.9	245.6	245.1	245.1	240.4	253.7	435

TABLE 20*

KERTÉSZ'S TABLE FOR METHOD OF BERTRAND

Copper (Cu)	Glucose	Invert Sugar	Maltose, Anhydrous	Lactose, Anhydrous	Mannose	Galactose	Sorbose	Arabinose	Xylose	Glucuronic Acid	Galacturonic Acid
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1	0.4	....	....	....	....	....	....	....	....	....	....
2	0.9	....	....	....	....	....	....	....	....	....	....
3	1.4	....	....	....	....	....	....	....	....	....	....
4	1.8	....	....	....	....	....	....	....	....	....	....
5	2.3	....	....	....	....	....	....	....	....	....	....
6	2.8	....	....	....	....	....	....	....	....	....	....
7	3.3	....	....	....	....	....	....	....	....	....	....
8	3.8	....	....	....	....	....	....	....	....	....	....
9	4.3	....	....	....	....	....	....	....	....	....	....
10	4.8	....	....	....	....	....	....	....	....	....	....
11	5.3	....	....	....	....	....	....	....	....	....	....
12	5.8	....	10.7	....	....	....	....	....	....	....	....
13	6.3	....	11.6	....	....	....	....	....	....	....	....
14	6.8	....	12.5	....	....	....	....	....	....	....	10.4
15	7.2	....	13.5	10.4	....	....	....	....	....	....	11.1
16	7.7	....	14.4	11.1	....	....	10.4	....	....	....	11.8
17	8.2	....	15.3	11.9	....	....	11.1	....	....	....	12.6
18	8.7	....	16.2	12.6	....	....	11.7	....	....	....	13.3
19	9.2	....	17.1	13.3	....	....	12.4	....	....	10.0	14.0
20	9.8	....	18.0	14.0	....	10.4	13.0	....	....	10.6	14.7
21	10.3	10.2	19.0	14.7	10.1	10.9	13.7	....	10.4	11.2	15.4
22	10.8	10.7	19.8	15.4	10.6	11.4	14.3	10.4	10.9	11.8	16.1
23	11.3	11.2	20.7	16.2	11.1	12.0	15.0	10.9	11.5	12.3	16.9
24	11.9	11.7	21.7	16.9	11.6	12.5	15.7	11.4	12.0	12.8	17.6
25	12.4	12.2	22.6	17.6	12.1	13.1	16.4	11.8	12.5	13.4	18.3
26	12.9	12.7	23.5	18.3	12.6	13.6	17.0	12.3	13.0	14.0	19.0
27	13.3	13.2	24.3	19.0	13.1	14.2	17.7	12.8	13.5	14.5	19.7
28	13.8	13.7	25.3	19.7	13.7	14.7	18.3	13.3	14.0	15.0	20.4
29	14.4	14.2	26.1	20.5	14.2	15.2	19.0	13.8	14.5	15.5	21.2
30	14.9	14.8	27.0	21.2	14.7	15.8	19.7	14.2	15.1	16.0	21.9
31	15.4	15.3	27.9	22.0	15.2	16.3	20.3	14.7	15.6	16.6	22.6
32	15.9	15.8	28.8	22.6	15.7	16.8	21.0	15.2	16.1	17.2	23.3
33	16.4	16.3	29.7	23.4	16.2	17.4	21.7	15.7	16.6	17.8	24.0
34	16.9	16.8	30.6	24.1	16.7	17.9	22.3	16.2	17.1	18.4	24.7
35	17.4	17.3	31.5	24.9	17.2	18.4	23.0	16.7	17.6	19.0	25.5
36	17.9	17.8	32.5	25.6	17.7	19.0	23.7	17.1	18.1	19.5	26.2
37	18.4	18.3	33.4	26.3	18.2	19.5	24.4	17.6	18.7	20.0	27.0
38	18.9	18.8	34.4	27.0	18.7	20.1	25.1	18.1	19.2	20.6	27.8
39	19.4	19.3	35.3	27.7	19.2	20.6	25.7	18.6	19.7	21.2	28.5
40	20.0	19.8	36.2	28.5	19.7	21.1	26.4	19.1	20.2	21.7	29.2

* Taken, with the permission of the author, from Z. I. Kertész, "Recalculated Tables for the Determination of Reducing Sugars by Bertrand's Method," Geneva, N. Y., 1930, and from article by Kertész in *J. Biol. Chem.*, **108**, 127 (1935). The original tables are in steps of 0.2 mg. copper. See text, p. 802.

TABLE 20 (*Continued*)

Copper (Cu)	Glucose	Invert Sugar	Maltose, Anhydrous	Lactose, Anhydrous	Mannose	Galactose	Sorbose	Arabinose	Xylose	Glucuronic Acid	Galacturonic Acid
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
41	20.5	20.3	37.1	29.2	20.3	21.7	27.1	19.6	20.7	22.3	29.3
42	21.0	20.8	38.1	29.9	20.8	22.2	27.8	20.0	21.2	22.8	30.0
43	21.5	21.4	39.0	30.7	21.3	22.8	28.4	20.5	21.8	23.4	30.8
44	22.1	21.9	39.9	31.4	21.8	23.3	29.1	21.0	22.3	24.0	31.5
45	22.6	22.4	40.8	32.2	22.4	23.9	29.8	21.5	22.8	24.5	32.3
46	23.1	22.9	41.7	32.9	22.9	24.5	30.5	22.0	23.3	25.1	33.0
47	23.6	23.5	42.6	33.7	23.4	25.0	31.2	22.5	23.9	25.6	33.8
48	24.2	24.0	43.5	34.5	23.9	25.5	31.9	23.0	24.4	26.2	34.5
49	24.7	24.5	44.5	35.2	24.4	26.1	32.5	23.5	24.9	26.7	35.3
50	25.2	25.1	45.4	35.9	25.0	26.6	33.2	24.0	25.4	27.3	36.0
51	25.8	25.6	46.3	36.7	25.5	27.2	33.9	24.5	26.0	27.9	36.8
52	26.3	26.2	47.3	37.5	26.0	27.8	34.6	25.0	26.5	28.5	37.6
53	26.8	26.7	48.2	38.2	26.5	28.4	35.3	25.5	27.0	29.0	38.3
54	27.3	27.2	49.1	39.0	27.1	28.9	36.0	26.0	27.5	29.6	39.0
55	27.8	27.7	50.0	39.7	27.6	29.5	36.7	26.5	28.1	30.1	39.8
56	28.4	28.2	51.0	40.5	28.1	30.0	37.4	27.0	28.6	30.7	40.6
57	28.9	28.8	51.9	41.3	28.7	30.6	38.0	27.5	29.1	31.3	41.3
58	29.4	29.3	52.8	42.0	29.2	31.1	38.7	28.0	29.6	31.8	42.0
59	30.0	29.8	53.8	42.8	29.7	31.7	39.4	28.5	30.2	32.3	42.8
60	30.5	30.4	54.8	43.5	30.3	32.2	40.1	29.0	30.7	32.9	43.6
61	31.0	30.9	55.7	44.3	30.8	32.7	40.7	29.5	31.2	33.5	44.3
62	31.6	31.4	56.6	45.1	31.3	33.3	41.5	30.0	31.8	34.1	45.0
63	32.1	32.0	57.5	45.8	31.9	33.9	42.2	30.5	32.3	34.5	45.8
64	32.7	32.5	58.4	46.6	32.4	34.4	42.9	31.0	32.9	35.1	46.6
65	33.2	33.1	59.4	47.3	33.0	35.0	43.5	31.6	33.4	35.7	47.3
66	33.7	33.6	60.3	48.1	33.5	35.5	44.2	32.1	33.9	36.2	48.0
67	34.3	34.2	61.2	48.9	34.0	36.1	44.9	32.6	34.5	36.8	48.8
68	34.8	34.7	62.1	49.7	34.6	36.7	45.6	33.1	35.0	37.4	49.6
69	35.4	35.2	63.1	50.4	35.1	37.2	46.3	33.6	35.5	38.0	50.3
70	35.9	35.8	64.0	51.2	35.7	37.8	47.0	34.1	36.1	38.7	51.1
71	36.5	36.3	64.9	52.0	36.2	38.4	47.7	34.6	36.6	39.3	51.9
72	37.0	36.9	65.8	52.7	36.7	38.9	48.5	35.1	37.2	39.9	52.7
73	37.6	37.4	66.8	53.5	37.3	39.5	49.2	35.6	37.7	40.4	53.5
74	38.1	38.0	67.7	54.3	37.8	40.1	49.9	36.1	38.3	41.0	54.2
75	38.6	38.5	68.7	55.1	38.4	40.7	50.6	36.7	38.8	41.6	55.0
76	39.2	39.1	69.6	55.8	38.9	41.2	51.3	37.2	39.3	42.2	55.8
77	39.7	39.6	70.5	56.6	39.5	41.8	52.0	37.7	39.9	42.8	56.7
78	40.3	40.2	71.4	57.4	40.0	42.4	52.7	38.2	40.4	43.4	57.5
79	40.8	40.7	72.4	58.1	40.6	43.0	53.4	38.7	40.9	44.0	58.3
80	41.4	41.3	73.3	59.0	41.1	43.5	54.1	39.3	41.5	44.6	59.1
81	41.9	41.8	74.2	59.8	41.7	44.1	54.8	39.8	42.0	45.2	60.0
82	42.5	42.4	75.1	60.6	42.2	44.7	55.5	40.3	42.6	45.8	60.7
83	43.0	43.0	76.0	61.3	42.8	45.3	56.2	40.8	43.1	46.3	61.5
84	43.6	43.5	77.0	62.1	43.3	45.9	56.9	41.3	43.7	46.9	62.3
85	44.2	44.1	77.9	62.9	43.9	46.4	57.6	41.8	44.2	47.5	63.2

TABLE 20 (*Continued*)

Copper (Cu)	Glucose	Invert Sugar	Maltose, Anhydrous	Lactose, Anhydrous	Mannose	Galactose	Sorbose	Arabinose	Xylose	Glucuronic Acid	Galacturonic Acid
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
86	44.7	44.7	78.9	63.7	44.5	47.0	58.3	42.4	44.8	48.2	64.0
87	45.3	45.2	79.8	64.4	45.0	47.6	59.0	42.9	45.3	48.7	64.8
88	45.9	45.8	80.7	65.2	45.6	48.2	59.7	43.4	45.9	49.3	65.6
89	46.4	46.4	81.7	66.0	46.1	48.7	60.4	43.9	46.5	50.0	66.5
90	47.0	46.9	82.6	66.8	46.7	49.3	61.1	44.4	47.0	50.6	67.3
91	47.6	47.5	83.5	67.6	47.3	49.9	61.9	44.9	47.6	51.2	68.0
92	48.1	48.1	84.5	68.4	47.8	50.5	62.6	45.5	48.1	52.0	68.8
93	48.7	48.6	85.4	69.2	48.4	51.1	63.2	46.0	48.7	52.8	69.7
94	49.2	49.2	86.3	69.9	48.9	51.7	64.0	46.5	49.2	53.5	70.5
95	49.8	49.8	87.2	70.7	49.5	52.2	64.7	47.0	49.8	54.2	71.3
96	50.3	50.3	88.2	71.5	50.1	52.8	65.4	47.6	50.3	55.0	72.1
97	50.9	50.9	89.1	72.3	50.6	53.4	66.2	48.1	50.9	55.7	72.9
98	51.5	51.5	90.0	73.1	51.2	54.0	66.9	48.6	51.4	56.4	73.7
99	52.1	52.1	91.0	73.9	51.8	54.6	67.6	49.2	52.0	57.2	74.5
100	52.6	52.6	91.9	74.7	52.3	55.2	68.3	49.7	52.6	57.8	75.3
101	53.2	53.2	92.9	75.5	52.9	55.8	69.1	50.2	53.1	58.6	76.2
102	53.8	53.8	93.8	76.3	53.5	56.3	69.8	50.8	53.7	59.2	77.0
103	54.4	54.4	94.8	77.1	54.1	56.9	70.5	51.3	54.2	60.0	77.7
104	54.9	55.0	95.8	77.9	54.7	57.5	71.2	51.8	54.8	60.7	78.5
105	55.5	55.6	96.8	78.7	55.2	58.1	72.0	52.3	55.4	61.5	79.3
106	56.1	56.2	97.7	79.4	55.8	58.6	72.7	52.9	55.9	62.3	80.2
107	56.7	56.8	98.7	80.3	56.4	59.2	73.4	53.4	56.5	63.0	81.0
108	57.2	57.3	99.6	81.1	57.0	59.8	74.2	53.9	57.1	63.8	81.8
109	57.8	57.9	....	81.8	57.5	60.4	74.9	54.5	57.6	64.5	82.5
110	58.4	58.5	....	82.6	58.1	61.0	75.6	55.0	58.2	65.2	83.3
111	59.0	59.1	....	83.4	58.7	61.6	76.4	55.5	58.8	65.8	84.2
112	59.5	59.7	....	84.2	59.2	62.2	77.1	56.1	59.3	66.6	85.0
113	60.1	60.4	....	85.0	59.8	62.8	77.8	56.6	59.9	67.3	85.8
114	60.7	60.9	....	85.9	60.4	63.4	78.6	57.2	60.4	68.0	86.6
115	61.3	61.4	....	86.7	61.0	64.0	79.3	57.7	61.0	68.7	87.4
116	61.9	62.0	....	87.5	61.6	64.6	80.1	58.2	61.6	69.5	88.2
117	62.5	62.6	....	88.3	62.2	65.2	80.8	58.8	62.2	70.2	89.0
118	63.0	63.3	....	89.1	62.8	65.8	81.5	59.3	62.8	71.0	89.9
119	63.6	63.9	....	89.9	63.4	66.4	82.3	59.8	63.3	71.6	90.7
120	64.2	64.4	....	90.7	64.0	67.0	83.0	60.4	63.9	72.3	91.5
121	64.8	65.0	....	91.5	64.6	67.6	83.7	60.9	64.5	73.0	92.3
122	65.4	65.6	....	92.4	65.2	68.2	84.5	61.5	65.0	73.7	93.0
123	66.0	66.2	....	93.2	65.7	68.8	85.2	62..	65 6	74.5	93.8
124	66.6	66.8	....	94.0	66.3	69.4	86.0	62.6	66 2	75.2	94.7
125	67.2	67.4	....	94.8	66.9	70.0	86.7	63.1	66	75 8	95.5
126	67.8	68.1	....	95.6	67.5	70.6	87.5	63.7	67.4	76.6	96.3
127	68.4	68.7	....	96.4	68.1	71.2	88.2	64.2	67.9	77.3	97.2
128	69.0	69.3	....	97.2	68.7	71.8	89.0	64.7	68.5	78.0	98.0
129	69.5	69.9	....	98.0	69.3	72.4	89.7	65.3	69.1	78.7	98.8
130	70.1	70.5	....	98.9	69.9	73.0	90.4	65.8	69.7	79.5	99.6

TABLE 20 (*Continued*)

Copper (Cu)	Glucose	Invert Sugar	Maltose, Anhydrous	Lactose, Anhydrous	Mannose	Galactose	Sorbose	Arabinose	Xylose	Glucuronic Acid	Galacturonic Acid
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
131	70.7	71.1	....	99.7	70.5	73.7	91.2	66.4	70.2	80.2	100.4
132	71.3	71.8	....	....	71.1	74.3	91.9	67.0	70.8	80.9	.....
133	72.0	72.4	....	....	71.7	74.9	92.7	67.5	71.4	81.6	.....
134	72.6	73.0	....	....	72.3	75.5	93.4	68.1	72.0	82.3	.....
135	73.2	73.6	....	....	72.9	76.1	94.2	68.6	72.5	83.0	.....
136	73.8	74.2	....	....	73.5	76.7	94.9	69.2	73.1	83.7	.....
137	74.4	74.9	....	....	74.1	77.3	95.7	69.7	73.7	84.5	.....
138	75.0	75.5	....	....	74.7	78.0	96.4	70.3	74.3	85.2	.....
139	75.6	76.1	....	....	75.3	78.6	97.2	70.8	74.9	85.9	.....
140	76.3	76.7	....	....	75.9	79.2	97.9	71.4	75.5	86.6	.....
141	76.9	77.3	....	....	76.5	79.8	98.7	72.0	76.1	87.3	.....
142	77.5	77.9	....	....	77.1	80.4	99.4	72.5	76.7	88.0	.....
143	78.1	78.6	....	....	77.7	81.0	....	73.1	77.2	88.7	.....
144	78.7	79.2	....	....	78.3	81.7	....	73.6	77.8	89.5	.....
145	79.3	79.8	....	....	78.9	82.3	....	74.2	78.4	90.2	.....
146	80.0	80.4	....	....	79.5	82.9	....	74.8	79.0	91.0	.....
147	80.6	81.0	....	....	80.1	83.5	....	75.3	79.6	91.7	.....
148	81.2	81.7	....	....	80.7	84.1	....	75.9	80.2	92.3	.....
149	81.8	82.3	....	....	81.3	84.7	....	76.4	80.8	93.1	.....
150	82.4	83.0	....	....	81.9	85.3	....	77.0	81.4	93.8	.....
151	83.0	83.6	....	....	82.5	85.9	....	77.6	82.0	94.5	.....
152	83.7	84.2	....	....	83.1	86.6	....	78.1	82.7	95.3	.....
153	84.3	84.9	....	....	83.7	87.2	....	78.7	83.3	96.0	.....
154	85.0	85.5	....	....	84.3	87.8	....	79.3	83.9	96.7	.....
155	85.6	86.1	....	....	84.9	88.4	....	79.8	84.4	97.3	.....
156	86.2	86.7	....	....	85.5	89.0	....	80.4	85.0	98.1	.....
157	86.8	87.4	....	....	86.2	89.6	....	81.0	85.6	98.8	.....
158	87.5	88.0	....	....	86.8	90.3	....	81.6	86.3	99.5	.....
159	88.1	88.6	....	....	87.4	90.9	....	82.1	86.9	100.2	.....
160	88.7	89.3	....	....	88.0	91.5	....	82.7	87.5	.....	.....
161	89.4	89.9	....	....	88.6	92.1	....	83.3	88.1	.....	.....
162	90.0	90.6	....	....	89.2	92.8	....	83.8	88.7	.....	.....
163	90.6	91.2	....	....	89.8	93.4	....	84.4	89.3	.....	.....
164	91.3	91.9	....	....	90.4	94.0	....	85.0	89.9	.....	.....
165	91.9	92.5	....	....	91.1	94.6	....	85.6	90.5	.....	.....
166	92.5	93.2	....	....	91.7	95.2	....	86.1	91.1	.....	.....
167	93.2	93.8	....	....	92.3	95.9	....	86.7	91.7	.....	.....
168	93.8	94.5	....	....	92.9	96.5	....	87.3	92.3	.....	.....
169	94.4	95.2	....	....	93.5	97.1	....	87.9	92.9	.....	.....
170	95.0	95.8	....	....	94.2	97.7	....	88.4	93.5	.....	.....
171	95.7	96.4	....	....	94.8	98.4	....	89.0	94.2	.....	.....
172	96.3	97.1	....	....	95.4	99.0	....	89.6	94.8	.....	.....
173	97.0	97.7	....	....	96.0	99.6	....	90.2	95.4	.....	.....
174	97.6	98.4	....	....	96.7	....	....	90.8	96.0	.....	.....
175	98.2	99.0	....	....	97.3	....	....	91.4	96.6	.....	.....

TABLE 20 (*Concluded*)

Copper (Cu)	Glucose	Invert Sugar	Maltose, Anhydrous	Lactose, Anhydrous	Mannose	Galactose	Sorbose	Arabinose	Xylose	Glucuronic Acid	Galacturonic Acid
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
176	98.9	99.7	....	....	97.9	....	....	91.9	97.2	.....	.....
177	99.5	....	....	....	98.5	....	....	92.5	97.8	.....	.....
178	....	....	....	....	99.2	....	....	93.1	98.5	.....	.....
179	....	....	....	....	99.7	....	....	93.7	99.1	.....	.....
180	....	....	....	....	....	....	....	94.3	99.7	.....	.....
181	....	....	....	....	....	....	....	94.8	....	.....	.....
182	....	....	....	....	....	....	....	95.4	....	.....	.....
183	....	....	....	....	....	....	....	96.0	....	.....	.....
184	....	....	....	....	....	....	....	96.6	....	.....	.....
185	....	....	....	....	....	....	....	97.2	....	.....	.....
186	....	....	....	....	....	....	....	97.8	....	.....	.....
187	....	....	....	....	....	....	....	98.4	....	.....	.....
188	....	....	....	....	....	....	....	99.0	....	.....	.....
189	....	....	....	....	....	....	....	99.5	....	.....	.....

TABLE 21*

Quisumbing and Thomas's Table for Determining Glucose, Fructose, Invert Sugar, Lactose, and Maltose

Expressed in milligrams

Copper (Cu)	Cuprous Oxide (Cu_2O)	Glucose	Fructose	Invert Sugar	Lactose		Maltose	
					$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}$ H_2O	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}$ H_2O
10	11.1	4.8	5.3	5.0	7.7	8.1	9.4	9.9
20	22.5	9.5	10.5	10.1	15.5	16.3	18.8	19.8
30	33.8	14.3	15.8	15.2	23.2	24.4	28.2	29.7
40	45.0	19.1	21.2	20.3	30.9	32.5	37.6	39.6
50	56.3	24.0	26.5	25.4	38.7	40.7	47.0	49.5
60	67.6	28.9	31.9	30.6	46.4	48.8	56.4	59.4
70	78.8	33.7	37.2	35.7	54.0	56.9	65.8	69.3
80	90.1	38.7	42.6	40.9	61.7	65.0	75.2	79.2
90	101.3	43.6	48.0	46.1	69.5	73.2	84.6	89.1
100	112.6	48.6	53.4	51.3	77.2	81.3	94.0	99.0
110	123.8	53.5	58.8	56.5	85.0	89.5	103.4	108.9
120	135.1	58.5	64.3	61.8	92.7	97.0	112.8	118.8
130	146.4	63.6	70.7	67.0	100.4	105.7	122.2	128.7
140	157.6	68.6	75.2	72.3	108.2	113.9	131.6	138.6
150	168.9	73.7	80.7	77.6	116.0	122.0	141.0	148.5
160	180.1	78.8	86.2	82.9	123.7	130.1	150.4	158.4
170	191.4	83.9	91.7	88.3	131.4	138.3	159.8	168.3
180	202.6	89.1	97.2	93.7	139.1	146.4	169.2	178.2
190	213.9	94.2	102.8	99.1	146.9	154.6	178.8	188.1
200	225.2	99.4	108.4	104.4	154.6	162.7	188.2	198.0
210	236.4	104.6	114.0	109.8	162.3	170.9	197.6	207.9
220	247.7	109.9	119.6	115.2	170.0	179.0	207.0	217.8
230	258.9	115.1	125.2	120.6	177.8	187.2	216.4	227.7
240	270.2	120.4	130.8	126.1	185.5	195.3	225.8	237.6
250	281.5	125.7	136.4	131.6	193.2	203.4	235.2	247.5
260	292.7	131.0	142.1	137.1	201.0	211.6	244.6	257.4
270	304.0	136.4	147.8	142.6	208.8	219.8	254.0	267.3
280	315.2	141.7	153.5	148.2	216.5	227.9	263.4	277.2
290	326.5	147.1	159.2	153.7	224.2	236.0	272.8	287.1
300	337.8	152.6	165.0	159.3	232.0	244.2	282.2	297.0
310	349.0	158.0	170.7	164.9	239.7	252.3	291.6	306.9
320	360.3	163.5	176.5	170.5	247.5	260.5	301.0	316.8
330	371.5	168.9	182.3	176.1	255.3	268.7	310.4	326.7
340	382.8	174.5	188.1	181.8	263.0	276.8	319.8	336.6
350	394.0	180.0	193.9	187.4	270.7	285.0	329.2	346.5
360	405.3	185.5	199.7	193.1	278.4	293.1	338.6	356.4
370	416.6	191.1	205.5	198.8	286.2	301.3	348.0	366.3
380	427.8	196.7	211.4	204.5	293.9	309.4	357.4	376.2
390	439.1	202.3	217.3	210.2	301.6	317.5	366.8	386.1
400	450.3	208.0	223.2	216.0	309.4	325.7	376.2	396.0
410	461.6	213.7	229.1	221.8	317.1	333.8	385.6	405.9
420	472.9	219.4	235.0	227.6	324.9	342.0	395.0	415.8
430	484.1	225.1	240.9	233.4	332.6	350.1	404.4	425.7
440	495.4	230.8	246.9	239.2	340.4	358.3	413.8	435.6
450	506.6	236.6	252.9	245.0	348.1	366.4	423.2	445.5
460	517.9	242.4	258.9	250.9	355.9	374.6	432.6	455.4
470	529.1	248.1	264.9	256.8	363.6	382.7	442.0	465.3
480	540.4	250.8	270.9	262.7	371.3	390.9	451.4	475.2

* See text, p. 803. Taken from *J. Am. Chem. Soc.*, **43**, 1522 (1921).

TABLE 22*

Herzfeld's Table for Determining Invert Sugar in Raw Sugars (Invert Sugar Not to Exceed 1.5 Per Cent)

Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar
mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent
50	0.050	101	0.305	152	0.574	203	0.863
51	0.054	102	0.310	153	0.580	204	0.869
52	0.058	103	0.315	154	0.586	205	0.874
53	0.062	104	0.320	155	0.592	206	0.880
54	0.066	105	0.325	156	0.598	207	0.885
55	0.070	106	0.330	157	0.604	208	0.891
56	0.074	107	0.335	158	0.609	209	0.896
57	0.078	108	0.340	159	0.615	210	0.902
58	0.082	109	0.346	160	0.621	211	0.907
59	0.086	110	0.351	161	0.627	212	0.913
60	0.090	111	0.356	162	0.633	213	0.918
61	0.094	112	0.361	163	0.639	214	0.924
62	0.098	113	0.366	164	0.645	215	0.929
63	0.103	114	0.371	165	0.651	216	0.935
64	0.108	115	0.376	166	0.657	217	0.940
65	0.113	116	0.381	167	0.663	218	0.946
66	0.118	117	0.386	168	0.669	219	0.951
67	0.123	118	0.392	169	0.675	220	0.957
68	0.128	119	0.397	170	0.680	221	0.962
69	0.133	120	0.402	171	0.686	222	0.968
70	0.138	121	0.407	172	0.692	223	0.973
71	0.143	122	0.412	173	0.698	224	0.979
72	0.148	123	0.417	174	0.704	225	0.984
73	0.152	124	0.423	175	0.709	226	0.990
74	0.157	125	0.428	176	0.715	227	0.996
75	0.162	126	0.433	177	0.720	228	1.001
76	0.167	127	0.438	178	0.726	229	1.007
77	0.172	128	0.443	179	0.731	230	1.013
78	0.177	129	0.448	180	0.737	231	1.018
79	0.182	130	0.453	181	0.742	232	1.024
80	0.187	131	0.458	182	0.748	233	1.030
81	0.192	132	0.463	183	0.753	234	1.036
82	0.197	133	0.468	184	0.759	235	1.041
83	0.202	134	0.473	185	0.764	236	1.047
84	0.208	135	0.478	186	0.770	237	1.053
85	0.213	136	0.483	187	0.775	238	1.058
86	0.219	137	0.488	188	0.781	239	1.064
87	0.225	138	0.493	189	0.786	240	1.070
88	0.231	139	0.498	190	0.792	241	1.076
89	0.236	140	0.503	191	0.797	242	1.081
90	0.242	141	0.509	192	0.803	243	1.087
91	0.248	142	0.515	193	0.808	244	1.093
92	0.254	143	0.521	194	0.814	245	1.099
93	0.260	144	0.527	195	0.819	246	1.104
94	0.265	145	0.533	196	0.825	247	1.110
95	0.271	146	0.538	197	0.830	248	1.116
96	0.277	147	0.544	198	0.836	249	1.122
97	0.283	148	0.550	199	0.841	250	1.127
98	0.288	149	0.556	200	0.847	251	1.133
99	0.294	150	0.562	201	0.852	252	1.139
100	0.300	151	0.568	202	0.858	253	1.144

* See text, p. 807. Taken from *Z. Ver. deut. Zucker-Ind.*, 35, 1012 (1885), and interpolated in steps of 1 mg.

TABLE 22 (*Concluded*)

Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar	Copper (Cu)	Invert Sugar
mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent
254	1.150	270	1.242	286	1.334	302	1.425
255	1.156	271	1.248	287	1.339	303	1.431
256	1.162	272	1.253	288	1.345	304	1.437
257	1.167	273	1.259	289	1.351	305	1.443
258	1.173	274	1.265	290	1.357	306	1.448
259	1.179	275	1.271	291	1.362	307	1.454
260	1.185	276	1.276	292	1.368	308	1.460
261	1.190	277	1.282	293	1.374	309	1.466
262	1.196	278	1.288	294	1.380	310	1.471
263	1.202	279	1.294	295	1.385	311	1.477
264	1.207	280	1.299	296	1.391	312	1.483
265	1.213	281	1.305	297	1.397	313	1.489
266	1.219	282	1.311	298	1.403	314	1.494
267	1.225	283	1.317	299	1.408	315	1.500
268	1.231	284	1.322	300	1.414	....	.....
269	1.236	285	1.328	301	1.420	....	.....

TABLE 23*

Baumann's Table for Determining Invert Sugar in Raw Sugars

Using 5 g. of sugar

Copper	Invert Sugar	Copper	Invert Sugar	Copper	Invert Sugar
mg.	per cent	mg.	per cent	mg.	per cent
(35)	(0.04)	135	1.10	230	2.16
40	0.09	140	1.15	235	2.21
45	0.14	145	1.21	240	2.27
50	0.19	150	1.26	245	2.33
55	0.25	155	1.31	250	2.39
60	0.30	160	1.37	255	2.44
65	0.35	165	1.42	260	2.50
70	0.40	170	1.48	265	2.56
75	0.45	175	1.54	270	2.62
80	0.51	180	1.59	275	2.68
85	0.56	185	1.65	280	2.74
90	0.61	190	1.70	285	2.79
95	0.66	195	1.76	290	2.85
100	0.72	200	1.82	295	2.91
105	0.77	205	1.87	300	2.97
110	0.83	210	1.93	305	3.03
115	0.88	215	1.98	310	3.09
120	0.93	220	2.04	315	3.15
125	0.99	225	2.10	320	3.21
130	1.04	...	....	...	....

* See text, p. 807. Taken from *Z. Ver. deut. Zucker-Ind.*, **42**, 826 (1892).

TABLE 24*

Schrefeld's Table for Determining Invert Sugar in Beet Molasses

Copper	Invert Sugar	Copper	Invert Sugar	Copper	Invert Sugar
mg.	per cent	mg.	per cent	mg.	per cent
27	0.05	125	1.09	225	2.22
30	0.08	130	1.14	230	2.28
35	0.13	135	1.20	235	2.34
40	0.18	140	1.25	240	2.40
45	0.24	145	1.31	245	2.46
50	0.29	150	1.37	250	2.52
55	0.34	155	1.42	255	2.57
60	0.39	160	1.48	260	2.63
65	0.44	165	1.53	265	2.69
70	0.50	170	1.59	270	2.75
75	0.55	175	1.65	275	2.81
80	0.60	180	1.70	280	2.87
85	0.66	185	1.76	285	2.94
90	0.71	190	1.82	290	3.00
95	0.76	195	1.87	295	3.06
100	0.82	200	1.93	300	3.12
105	0.87	205	1.99	305	3.18
110	0.93	210	2.05	310	3.24
115	0.98	215	2.10	315	3.30
120	1.03	220	2.16	320	3.36

* See text, p. 807. Taken from *Z. Ver. deut. Zucker-Ind.*, **61**, 988 (1911).

TABLE 25*

Saillard's Table for Determining Invert Sugar in the Presence of Sucrose

Invert Sugar	Grams Sucrose in 50 ml. of Solution										
	0	0.815	1.630	2.440	3.620	4.070	4.890	5.700	6.520	7.330	8.150
mg.	Milligrams copper found										
0	0	0.8	1.6	2.5	3.2	3.8	4.1	4.4	4.7	5.0	5.2
4	7.0	8.0	8.7	9.5	10.2	10.8	11.2	11.5	11.8	12.1	12.4
8	14.0	15.2	16.1	16.8	17.5	18.1	18.6	19.0	19.4	19.8	20.1
12	21.0	22.4	23.2	24.0	24.7	25.3	25.8	26.3	26.8	27.2	27.6
16	28.0	29.6	30.4	31.2	31.9	32.5	33.1	33.7	34.2	34.7	35.2
20	35.0	36.8	37.6	38.4	39.1	39.7	40.3	41.0	41.6	42.2	42.8
24	42.0	44.0	44.8	45.6	46.3	46.9	47.6	48.3	49.0	49.7	50.4
28	49.0	51.2	52.0	52.8	53.5	54.1	54.8	55.6	56.4	57.2	58.0
32	56.0	58.4	59.2	60.0	60.7	61.3	62.1	63.0	63.9	64.8	65.6
36	63.0	65.6	66.4	67.2	67.9	68.5	69.4	70.4	71.3	72.3	73.2
40	70.0	72.8	73.6	74.4	75.1	75.9	76.8	77.8	78.8	79.8	80.8
44	77.0	80.0	80.8	81.6	82.3	83.1	84.2	85.2	86.2	87.3	88.4
48	84.0	87.2	88.0	88.8	89.6	90.4	91.5	92.6	93.7	94.8	96.0
52	91.0	94.4	95.2	96.0	96.8	97.7	98.8	100.0	101.2	102.4	103.6
56	98.0	101.6	102.4	103.2	104.1	105.0	106.2	107.4	108.7	110.0	111.2
60	105.0	108.8	109.6	110.4	111.3	112.3	113.6	114.8	116.2	117.5	118.8
64	112.0	115.9	116.8	117.6	118.6	119.7	121.0	122.3	123.7	125.1	126.5
68	119.0	123.1	124.0	124 8	125.9	127.0	128.4	129.8	131.2	132.7	134.1
72	126.0	130.3	131.1	132.0	133.2	134.4	135.8	137.3	138.8	140.3	141.8
76	133.0	137.5	138.3	139.3	140.5	141.7	143.2	144.8	146.4	147.9	149.4
80	140.0	144.7	145.5	146.5	147.8	149.1	.....	.....	.....	.....	.....

* Taken from *Bull. assoc. chim. sucr. dist.*, **40**, 219 (1922/23). See text, p. 815.

TABLE 26*

Table of Edwards and Osborn for Determining Invert Sugar in Beet Products

Part I

Showing Percentage of Invert Sugar in Sugars and Thick Juice by Quisumbing and Thomas Method, Using 5 g. of Dry Substance

Copper	Invert† Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance
mg.	per cent	mg.	per cent	mg.	per cent
25.0	0.00	.....	....	.....	....
26.8	0.02	81.6	0.62	136.4	1.22
28.7	0.04	83.5	0.64	138.3	1.24
30.5	0.06	85.3	0.66	140.1	1.26
32.3	0.08	87.1	0.68	141.9	1.28
34.1	0.10	88.9	0.70	143.7	1.30
36.0	0.12	90.8	0.72	145.6	1.32
37.8	0.14	92.6	0.74	147.4	1.34
39.6	0.16	94.4	0.76	149.2	1.36
41.4	0.18	96.2	0.78	151.0	1.38
43.3	0.20	98.1	0.80	152.9	1.40
45.1	0.22	99.9	0.82	154.7	1.42
46.9	0.24	101.7	0.84	156.5	1.44
48.7	0.26	103.5	0.86	158.3	1.46
50.6	0.28	105.4	0.88	160.2	1.48
52.4	0.30	107.2	0.90	162.0	1.50
54.2	0.32	109.0	0.92	163.8	1.52
56.1	0.34	110.9	0.94	165.7	1.54
57.9	0.36	112.7	0.96	167.5	1.56
59.7	0.38	114.5	0.98	169.3	1.58
61.5	0.40	116.3	1.00	171.1	1.60
63.4	0.42	118.2	1.02		
65.2	0.44	120.0	1.04		
67.0	0.46	121.8	1.06		
68.8	0.48	123.6	1.08		
70.7	0.50	125.5	1.10		
72.5	0.52	127.3	1.12		
74.3	0.54	129.1	1.14		
76.1	0.56	130.9	1.16		
78.0	0.58	132.8	1.18		
79.8	0.60	134.6	1.20		

* Taken from *Ind. Eng. Chem., Anal. Ed.*, 5, 42 (1933). See text, p. 816.

† Per cent invert sugar $= \frac{\text{mg. Cu} - 25.0}{91.333}$.

TABLE 26 (*Continued*)

Part II

Showing Percentage of Invert Sugar in Molasses by Quisumbing and Thomas Method, Using 5 g. of Dry Substance

Copper	Invert† Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance
mg.	per cent	mg.	per cent	mg.	per cent
18.0	0.00	....	....	.....	....
19.9	0.02	70.4	0.56	120.9	1.10
21.7	0.04	72.2	0.58	122.7	1.12
23.6	0.06	74.1	0.60	124.6	1.14
25.5	0.08	76.0	0.62	126.5	1.16
27.4	0.10	77.8	0.64	128.3	1.18
29.2	0.12	79.7	0.66	130.2	1.20
31.1	0.14	81.6	0.68	132.1	1.22
32.9	0.16	83.5	0.70	133.9	1.24
34.8	0.18	85.3	0.72	135.8	1.26
36.7	0.20	87.2	0.74	137.7	1.28
38.6	0.22	89.1	0.76	139.6	1.30
40.4	0.24	90.9	0.78	141.4	1.32
42.3	0.26	92.8	0.80	143.3	1.34
44.2	0.28	94.7	0.82	145.2	1.36
46.1	0.30	96.5	0.84	147.0	1.38
47.9	0.32	98.4	0.86	148.9	1.40
49.8	0.34	100.3	0.88	150.8	1.42
51.6	0.36	102.2	0.90	152.6	1.44
53.5	0.38	104.0	0.92	154.5	1.46
55.4	0.40	105.9	0.94	156.4	1.48
57.3	0.42	107.8	0.96	158.3	1.50
59.1	0.44	109.6	0.98	160.1	1.52
61.0	0.46	111.5	1.00	162.0	1.54
62.9	0.48	113.4	1.02	163.9	1.56
64.8	0.50	115.2	1.04	165.7	1.58
66.6	0.52	117.1	1.06	167.6	1.60
68.5	0.54	119.0	1.08		

† Per cent invert sugar $= \frac{\text{mg. Cu} - 18.0}{93.5}$.

TABLE 26 (*Continued*)

Part III

Showing Percentage of Invert Sugar in Molasses by Quisumbing and Thomas Method, Using 2.5 g. of Dry Substance

Copper	Invert† Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance
mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent
15.0	0.00	....	....	.....	....	.....	....
15.9	0.02	52.7	0.82	89.5	1.62	126.3	2.42
16.8	0.04	53.6	0.84	90.4	1.64	127.2	2.44
17.8	0.06	54.6	0.86	91.4	1.66	128.2	2.46
18.7	0.08	55.5	0.88	92.3	1.68	129.1	2.48
19.6	0.10	56.4	0.90	93.2	1.70	130.0	2.50
20.5	0.12	57.3	0.92	94.1	1.72	130.9	2.52
21.4	0.14	58.2	0.94	95.0	1.74	131.8	2.54
22.4	0.16	59.2	0.96	96.0	1.76	132.8	2.56
23.3	0.18	60.1	0.98	96.9	1.78	133.7	2.58
24.2	0.20	61.0	1.00	97.8	1.80	134.6	2.60
25.1	0.22	61.9	1.02	98.7	1.82	135.5	2.62
26.0	0.24	62.8	1.04	99.6	1.84	136.4	2.64
27.0	0.26	63.8	1.06	100.6	1.86	137.4	2.66
27.9	0.28	64.7	1.08	101.5	1.88	138.3	2.68
28.8	0.30	65.6	1.10	102.4	1.90	139.2	2.70
29.7	0.32	66.5	1.12	103.3	1.92	140.1	2.72
30.6	0.34	67.4	1.14	104.2	1.94	141.0	2.74
31.6	0.36	68.4	1.16	105.2	1.96	142.0	2.76
32.5	0.38	69.3	1.18	106.1	1.98	142.9	2.78
33.4	0.40	70.2	1.20	107.0	2.00	143.8	2.80
34.3	0.42	71.1	1.22	107.9	2.02	144.7	2.82
35.2	0.44	72.0	1.24	108.8	2.04	145.6	2.84
36.2	0.46	73.0	1.26	109.8	2.06	146.6	2.86
37.1	0.48	73.9	1.28	110.7	2.08	147.5	2.88
38.0	0.50	74.8	1.30	111.6	2.10	148.4	2.90
38.9	0.52	75.7	1.32	112.5	2.12	149.3	2.92
39.8	0.54	76.6	1.34	113.4	2.14	150.2	2.94
40.8	0.56	77.6	1.36	114.4	2.16	151.2	2.96
41.7	0.58	78.5	1.38	115.3	2.18	152.1	2.98
42.6	0.60	79.4	1.40	116.2	2.20	153.0	3.00
43.5	0.62	80.3	1.42	117.1	2.22	153.9	3.02
44.4	0.64	81.2	1.44	118.0	2.24	154.9	3.04
45.4	0.66	82.2	1.46	119.0	2.26	155.8	3.06
46.3	0.68	83.1	1.48	119.9	2.28	156.7	3.08
47.2	0.70	84.0	1.50	120.8	2.30	157.6	3.10
48.1	0.72	84.9	1.52	121.7	2.32	158.5	3.12
49.0	0.74	85.8	1.54	122.6	2.34	159.4	3.14
50.0	0.76	86.7	1.56	123.6	2.36	160.4	3.16
50.9	0.78	87.7	1.58	124.5	2.38	161.3	3.18
51.8	0.80	88.6	1.60	125.4	2.40	162.2	3.20

† Per cent invert sugar $= \frac{\text{mg. Cu} - 15.0}{46.0}$.

TABLE 26 (*Continued*)

Part IV

Showing Percentage of Invert Sugar in Sugars and Thick Juice by 2-Minute Boiling Method, Using 5 g. of Dry Substance

Copper	Invert† Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance
mg.	per cent	mg.	per cent	mg.	per cent
3.8	0.00	....	....	....	....
5.6	0.02	60.7	0.62	115.6	1.22
7.5	0.04	62.6	0.64	117.6	1.24
9.3	0.06	64.4	0.66	119.5	1.26
11.1	0.08	66.2	0.68	121.3	1.28
13.0	0.10	68.1	0.70	123.1	1.30
14.8	0.12	69.9	0.72	125.0	1.32
16.7	0.14	71.7	0.74	126.8	1.34
18.5	0.16	73.6	0.76	128.6	1.36
20.3	0.18	75.4	0.78	130.5	1.38
22.2	0.20	77.2	0.80	132.3	1.40
24.0	0.22	79.1	0.82	134.2	1.42
25.8	0.24	80.9	0.84	136.0	1.44
27.7	0.26	82.7	0.86	137.8	1.46
29.5	0.28	84.6	0.88	139.7	1.48
31.3	0.30	86.4	0.90	141.5	1.50
33.2	0.32	88.3	0.92	143.3	1.52
35.0	0.34	90.1	0.94	145.2	1.54
36.8	0.36	91.9	0.96	147.0	1.56
38.7	0.38	93.8	0.98	148.8	1.58
40.5	0.40	95.6	1.00	150.7	1.60
42.4	0.42	97.4	1.02	152.5	1.62
44.2	0.44	99.3	1.04	154.4	1.64
46.0	0.46	101.1	1.06	156.2	1.66
47.9	0.48	102.9	1.08	158.0	1.68
49.7	0.50	104.8	1.10	159.9	1.70
51.5	0.52	106.6	1.12	161.7	1.72
53.4	0.54	108.5	1.14	163.5	1.74
55.2	0.56	110.3	1.16	165.4	1.76
57.0	0.58	112.1	1.18	167.2	1.78
58.9	0.60	114.0	1.20	169.0	1.80

† **Per cent invert sugar** $= \frac{\text{mg. Cu} - 3.8}{91.80}$.

TABLE 26 (*Continued*)

Part V

Showing Percentage of Invert Sugar in Molasses by 2-Minute Boiling Method, Using 5 g. of Dry Substance

Copper	Invert† Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance
mg.	per cent	mg.	per cent	mg.	per cent
2.8	0.00	....	....	.....	....
4.6	0.02	59.1	0.62	113.5	1.22
6.4	0.04	60.9	0.64	115.3	1.24
8.2	0.06	62.7	0.66	117.1	1.26
10.1	0.08	64.5	0.68	119.0	1.28
11.9	0.10	66.3	0.70	120.8	1.30
13.7	0.12	68.1	0.72	122.6	1.32
15.5	0.14	70.0	0.74	124.4	1.34
17.3	0.16	71.8	0.76	126.2	1.36
19.1	0.18	73.6	0.78	128.0	1.38
21.0	0.20	75.4	0.80	129.9	1.40
22.8	0.22	77.2	0.82	131.7	1.42
24.6	0.24	79.0	0.84	133.5	1.44
26.4	0.26	80.8	0.86	135.3	1.46
28.2	0.28	82.7	0.88	137.1	1.48
30.0	0.30	84.5	0.90	138.9	1.50
31.8	0.32	86.3	0.92	140.7	1.52
33.7	0.34	88.1	0.94	142.6	1.54
35.5	0.36	89.9	0.96	144.4	1.56
37.3	0.38	91.7	0.98	146.2	1.58
39.1	0.40	93.6	1.00	148.0	1.60
40.9	0.42	95.4	1.02	149.8	1.62
42.7	0.44	97.2	1.04	151.6	1.64
44.5	0.46	99.0	1.06	153.4	1.66
46.4	0.48	100.8	1.08	155.3	1.68
48.2	0.50	102.6	1.10	157.1	1.70
50.0	0.52	104.4	1.12	158.9	1.72
51.8	0.54	106.3	1.14	160.7	1.74
53.6	0.56	108.1	1.16	162.5	1.76
55.4	0.58	109.9	1.18	164.3	1.78
57.3	0.60	111.7	1.20	166.2	1.80

† Per cent invert sugar $= \frac{\text{mg. Cu} - 2.8}{90.75}$.

TABLE 26 (*Concluded*)

Part VI

Showing Percentage of Invert Sugar in Molasses by 2-Minute Boiling Method, Using 2.5 g. of Dry Substance

Copper	Invert† Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance	Copper	Invert Sugar on Dry Substance
mg.	per cent	mg.	per cent	mg.	per cent
1.9	0.00	....	....	.....	....
3.7	0.04	57.7	1.24	111.8	2.44
5.5	0.08	59.5	1.28	113.6	2.48
7.3	0.12	61.3	1.32	115.4	2.52
9.1	0.16	63.1	1.36	117.2	2.56
10.9	0.20	64.9	1.40	119.0	2.60
12.7	0.24	66.7	1.44	120.8	2.64
14.5	0.28	68.5	1.48	122.6	2.68
16.3	0.32	70.3	1.52	124.4	2.72
18.1	0.36	72.1	1.56	126.2	2.76
19.9	0.40	73.9	1.60	128.0	2.80
21.7	0.44	75.7	1.64	129.8	2.84
23.5	0.48	77.6	1.68	131.6	2.88
25.3	0.52	79.4	1.72	133.4	2.92
27.1	0.56	81.2	1.76	135.2	2.96
28.9	0.60	83.0	1.80	137.0	3.00
30.7	0.64	84.8	1.84	138.8	3.04
32.5	0.68	86.6	1.88	140.6	3.08
34.3	0.72	88.4	1.92	142.4	3.12
36.1	0.76	90.2	1.96	144.2	3.16
37.9	0.80	92.0	2.00	146.0	3.20
39.7	0.84	93.8	2.04	147.8	3.24
41.5	0.88	95.6	2.08	149.6	3.28
43.3	0.92	97.4	2.12	151.4	3.32
45.1	0.96	99.2	2.16	153.2	3.36
46.9	1.00	101.0	2.20	155.0	3.40
48.7	1.04	102.8	2.24	156.8	3.44
50.5	1.08	104.6	2.28	158.6	3.48
52.3	1.12	106.4	2.32	160.4	3.52
54.1	1.16	108.2	2.36	162.2	3.56
55.9	1.20	110.0	2.40	164.0	3.60

† Per cent invert sugar $= \frac{\text{mg. Cu} - 1.9}{45.03}$.

TABLE 27*

FITELSON'S CORRECTION TABLE FOR DETERMINING GLUCOSE AND LACTOSE IN THE PRESENCE OF SUCROSE BY THE METHOD OF LANE AND EYNON

Glucose in the presence of sucrose
(Corrections in milliliters to be added to burette readings)

Burette Readings	Sucrose Glucose Ratios			
	2/1	4/1	8/1	20/1
	(a) for 10 ml. Soxhlet's Solution			
ml.				
15	0.20	0.40	0.65	1.15
20	0.20	0.45	0.65	1.15
25	0.25	0.45	0.70	1.25
30	0.30	0.50	0.75	1.45
35	0.35	0.55	0.90	1.75
40	0.40	0.60	1.10	2.15
45	0.45	0.65	1.35	2.60
50	0.55	0.70	1.60	3.15
	(b) for 25 ml. Soxhlet's Solution			
15	0.20	0.35	0.60	1.25
20	0.20	0.40	0.65	1.30
25	0.25	0.45	0.70	1.40
30	0.25	0.50	0.80	1.50
35	0.30	0.55	0.90	1.65
40	0.30	0.55	0.95	1.85
45	0.30	0.55	1.05	2.05
50	0.30	0.55	1.15	2.25

Lactose in the presence of sucrose
(Corrections in milliliters to be added to burette readings)

Burette Readings	Sucrose Lactose Ratios					
	3/1	6/1	10/1	12/1	15/1	20/1
	(a) 10 ml. Soxhlet's Solution					
ml.						
15	0.15	0.30	0.60	0.75	0.90	1.10
20	0.25	0.50	0.80	0.95	1.15	1.45
25	0.30	0.60	0.95	1.15	1.40	1.75
30	0.35	0.70	1.10	1.30	1.55	2.00
35	0.40	0.80	1.20	1.45	1.70	2.05
40	0.45	0.90	1.30	1.55	1.75	2.10
45	0.50	0.95	1.40	1.60	1.80	2.15
50	0.55	1.05	1.45	1.65	1.85	2.20
	(b) 25 ml. Soxhlet's Solution					
15	0.30	0.60	0.80	0.90	1.15	1.40
20	0.30	0.60	0.95	1.10	1.35	1.70
25	0.35	0.65	1.15	1.35	1.60	2.00
30	0.35	0.70	1.30	1.55	1.80	2.20
35	0.40	0.80	1.45	1.80	2.00	2.40
40	0.45	0.90	1.60	2.00	2.20	2.55
45	0.55	1.10	1.80	2.20	2.40	2.65
50	0.60	1.20	1.95	2.45	2.60	2.75

* Taken from *J. Assoc. Official Agr. Chem.*, 15, 625 (1932). See text, p. 818.

TABLE 28*

JACKSON AND MATHEWS'S TABLE FOR DETERMINING FRUCTOSE

All data expressed in milligrams

Cu	Fructose	Cu	Fructose	Cu	Fructose	Cu	Fructose	Cu	Fructose	Cu	Fructose	Cu	Fructose	Cu	Fructose
1	0.6	40	13.9	79	25.1	118	36.0	157	46.6	196	56.8	235	67.9	274	80.4
2	1.1	41	14.2	80	25.4	119	36.2	158	46.9	197	57.1	236	68.2	275	80.7
3	1.6	42	14.5	81	25.7	120	36.5	159	47.1	198	57.3	237	68.5	276	81.0
4	2.1	43	14.8	82	25.9	121	36.8	160	47.4	199	57.6	238	68.8	277	81.4
5	2.5	44	15.1	83	26.2	122	37.1	161	47.7	200	57.9	239	69.1	278	81.7
6	2.9	45	15.4	84	26.5	123	37.3	162	47.9	201	58.1	240	69.4	279	82.0
7	3.3	46	15.7	85	26.8	124	37.6	163	48.2	202	58.4	241	69.7	280	82.4
8	3.7	47	16.0	86	27.0	125	37.9	164	48.4	203	58.7	242	70.0	281	82.7
9	4.1	48	16.3	87	27.3	126	38.2	165	48.7	204	58.9	243	70.3	282	83.1
10	4.5	49	16.6	88	27.6	127	38.5	166	49.0	205	59.2	244	70.7	283	83.4
11	4.8	50	16.8	89	27.9	128	38.7	167	49.2	206	59.4	245	71.0	284	83.8
12	5.1	51	17.1	90	28.1	129	39.0	168	49.5	207	59.7	246	71.3	285	84.1
13	5.5	52	17.4	91	28.4	130	39.3	169	49.7	208	60.0	247	71.6	286	84.4
14	5.9	53	17.7	92	28.7	131	39.6	170	50.0	209	60.3	248	71.9	287	84.8
15	6.2	54	18.0	93	29.0	132	39.9	171	50.2	210	60.6	249	72.2	288	85.1
16	6.5	55	18.3	94	29.2	133	40.1	172	50.5	211	60.9	250	72.5	289	85.5
17	6.9	56	18.6	95	29.5	134	40.4	173	50.8	212	61.1	251	72.8	290	85.9
18	7.2	57	18.9	96	29.8	135	40.7	174	51.0	213	61.4	252	73.1	291	86.2
19	7.6	58	19.1	97	30.1	136	40.9	175	51.3	214	61.7	253	73.5	292	86.6
20	7.9	59	19.4	98	30.4	137	41.2	176	51.5	215	62.0	254	73.8	293	86.9
21	8.2	60	19.7	99	30.7	138	41.5	177	51.8	216	62.3	255	74.1	294	87.3
22	8.5	61	20.0	100	30.9	139	41.7	178	52.1	217	62.6	256	74.4	295	87.6
23	8.9	62	20.3	101	31.2	140	42.0	179	52.3	218	62.9	257	74.7	296	88.0
24	9.2	63	20.6	102	31.5	141	42.3	180	52.6	219	63.2	258	75.1	297	88.4
25	9.5	64	20.9	103	31.8	142	42.6	181	52.8	220	63.4	259	75.4	298	88.7
26	9.8	65	21.2	104	32.1	143	42.8	182	53.1	221	63.7	260	75.7	299	89.1
27	10.1	66	21.4	105	32.3	144	43.1	183	53.4	222	64.0	261	76.0	300	89.5
28	10.4	67	21.7	106	32.6	145	43.4	184	53.6	223	64.3	262	76.4	301	89.8
29	10.7	68	22.0	107	32.9	146	43.7	185	53.9	224	64.6	263	76.7	302	90.2
30	11.0	69	22.2	108	33.2	147	43.9	186	54.2	225	64.9	264	77.0	303	90.5
31	11.3	70	22.5	109	33.5	148	44.2	187	54.4	226	65.2	265	77.4	304	90.9
32	11.6	71	22.8	110	33.7	149	44.5	188	54.7	227	65.5	266	77.7	305	91.3
33	11.9	72	23.1	111	34.0	150	44.7	189	54.9	228	65.8	267	78.1	306	91.7
34	12.2	73	23.4	112	34.3	151	45.0	190	55.2	229	66.1	268	78.4	307	92.0
35	12.5	74	23.7	113	34.6	152	45.3	191	55.5	230	66.4	269	78.7	308	92.4
36	12.8	75	24.0	114	34.8	153	45.6	192	55.7	231	66.7	270	79.0	309	92.8
37	13.1	76	24.2	115	35.1	154	45.8	193	56.0	232	67.0	271	79.4	310	93 2
38	13.4	77	24.5	116	35.4	155	46.1	194	56.3	233	67.3	272	79.7	311	93.5
39	13.7	78	24.8	117	35.7	156	46.4	195	56.5	234	67.6	273	80.0	312	93.9

* See text, p. 825. Taken from *Bur. Standards J. Research.* 8, 440 (1932).

TABLE 29*

Schoorl's Table for Determining Glucose, Fructose, Invert Sugar, Galactose, Mannose, Arabinose, Xylose, and Rhamnose by Schoorl's Iodimetric Method

Using 20 ml. of Soxhlet's Solution

N/10 Thiosulfate	Glucose	Fructose	Invert Sugar	Galactose	Mannose	Arabinose	Xylose	Rhamnose
ml.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1	3.2	3.2	3.2	3.3	3.1	3.0	3.1	3.2
2	6.3	6.4	6.5	7.0	6.3	6.0	6.3	6.5
3	9.4	9.7	9.8	10.4	9.5	9.2	9.5	9.9
4	12.6	13.0	13.0	14.0	12.8	12.3	12.8	13.3
5	15.9	16.4	16.4	17.5	16.1	15.5	16.1	16.8
6	19.2	20.0	19.8	21.1	19.4	18.7	19.4	20.2
7	22.4	23.7	23.2	24.7	22.8	21.9	22.8	23.7
8	25.6	27.4	26.5	28.3	26.2	25.2	26.2	27.2
9	28.9	31.1	29.9	32.0	29.6	28.6	29.6	30.8
10	32.3	34.9	33.4	35.7	33.0	32.0	33.0	34.4
11	35.7	38.7	36.8	39.4	36.5	35.4	36.5	38.0
12	39.0	42.4	40.3	43.1	40.0	38.8	40.0	41.6
13	42.4	46.2	43.8	46.8	43.5	42.2	43.5	45.2
14	45.8	50.0	47.3	50.5	47.0	45.6	47.0	48.8
15	49.3	53.7	50.8	54.3	50.6	49.0	50.6	52.4
16	52.8	57.5	54.3	58.1	54.2	52.4	54.2	56.0
17	56.3	61.2	58.0	61.9	57.9	55.8	57.9	59.8
18	59.8	65.0	61.8	65.7	62.6	59.3	62.6	63.5
19	63.3	68.7	65.5	69.6	65.3	62.9	65.3	67.3
20	66.9	72.4	69.4	73.4	69.2	66.5	69.2	71.0
21	70.7	76.2	73.3	77.2	73.1	70.2	73.1	74.8
22	74.5	80.1	77.2	81.2	77.0	74.0	77.0	78.6
23	78.5	84.0	81.2	85.1	81.0	77.9	81.0	82.4
24	82.6	87.8	85.2	89.0	85.0	81.8	85.0	86.2
25	86.6	91.7	89.2	93.0	89.0	85.7	89.0	90.0
26	90.7	....	....	....	....	....	....	....
27	94.8	....	....	....	....	....	....	....

* Taken from van der Haar, " Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide," p. 122, and *Intern. Sugar J.*, **18**, 335 (1916). See text, p. 829.

TABLE 30*

Bruhns's Table for Determining Glucose, Fructose, Invert Sugar Alone, Invert Sugar in Presence of Sucrose, and Lactose

0.1387 *N* Thiosulfate	Glucose	Fructose	Invert Sugar, No Sucrose	Invert Sugar, 0.5 g. Sucrose	Invert Sugar, 1 g. Sucrose	Invert Sugar, 2 g. Sucrose	Invert Sugar, 4 g. Sucrose	Invert Sugar, 8 g. Sucrose	Lactose Hydrate
ml.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
0.1	0.9	0.9	0.9	....	....	....	....	....	0.8
0.2	1.2	1.3	1.2	....	....	....	....	....	1.5
0.3	1.6	1.8	1.7	0.1	....	....	....	....	2.3
0.4	2.0	2.3	2.2	0.5	....	....	....	....	3.0
0.5	2.5	2.8	2.7	0.9	....	....	....	....	3.7
0.6	2.9	3.4	3.2	1.4	0.2	....	....	....	4.4
0.7	3.4	3.8	3.7	1.8	0.7	....	....	....	5.2
0.8	3.8	4.3	4.1	2.2	1.1	....	....	....	5.9
0.9	4.3	4.8	4.6	2.7	1.6	....	....	....	6.7
1.0	4.7	5.3	5.1	3.2	2.1	....	....	....	7.4
1.1	5.2	5.8	5.5	3.6	2.5	0.5	....	....	8.2
1.2	5.6	6.3	6.0	4.1	2.9	1.0	....	....	8.9
1.3	6.1	6.8	6.5	4.5	3.3	1.5	....	....	9.6
1.4	6.5	7.3	7.0	5.0	3.7	1.9	0.5	0.5	10.3
1.5	7.0	7.8	7.4	5.5	4.1	2.3	1.0	1.0	11.0
2.0	9.2	10.3	9.7	7.9	6.2	4.4	3.4	3.0	14.5
2.5	11.5	12.8	12.1	10.3	8.5	6.7	5.4	5.0	18.0
3.0	13.7	15.2	14.5	12.6	10.7	9.1	7.7	7.0	21.4
3.5	16.0	17.6	16.8	14.9	13.1	11.4	10.0	9.0	24.9
4.0	18.2	20.1	19.2	17.2	15.6	13.8	12.3	11.1	28.4
4.5	20.5	22.6	21.5	19.6	18.0	16.1	14.6	13.3	31.8
5.0	22.8	25.0	23.8	21.9	20.4	18.5	16.8	15.4	35.3
5.5	25.0	27.5	26.2	24.2	22.8	20.8	19.1	17.6	38.8
6.0	27.3	30.0	28.5	26.5	25.2	23.2	21.4	19.8	42.2
6.5	29.7	32.5	30.9	28.8	27.5	25.5	23.7	21.9	45.7
7.0	32.0	35.0	33.4	31.2	29.7	28.0	26.1	24.1	49.2
7.5	34.4	37.5	35.8	33.7	32.2	30.4	28.6	26.3	52.6
8.0	36.7	40.0	38.3	36.2	34.7	32.9	31.1	28.7	56.1
8.5	39.1	42.5	40.7	38.6	37.2	35.4	33.6	31.1	59.6
9.0	41.4	45.0	43.2	41.1	39.7	37.9	36.0	33.5	63.0
9.5	43.7	47.5	45.6	43.6	42.1	40.4	38.4	35.8	66.5
10.0	46.1	50.0	48.0	46.1	44.6	42.8	40.8	38.2	70.0
10.5	48.4	52.6	50.5	48.6	47.1	45.3	43.2	40.6	73.4
11.0	50.8	55.1	53.1	51.1	49.6	47.8	45.7	43.0	76.9
11.5	53.3	57.7	55.6	53.7	52.2	50.3	48.1	45.3	80.4
12.0	55.9	60.3	58.1	56.3	54.8	52.9	50.6	47.7	83.8
12.5	58.4	62.9	60.7	58.9	57.5	55.6	53.2	50.0	87.3
13.0	60.9	65.4	63.2	61.4	60.1	58.2	55.7	52.5	90.8
13.5	63.5	68.0	65.8	64.0	62.7	60.8	58.3	54.9	94.2
14.0	66.0	70.6	68.3	66.6	65.3	63.4	60.8	57.4	97.7
14.5	68.5	73.2	70.8	69.2	68.0	66.0	63.4	59.8	101.2
15.0	71.1	75.7	73.4	71.8	70.6	68.7	66.0	62.2	104.6
15.5	73.6	....	76.0	74.4	73.2	71.3	68.6	64.7	108.1
16.0	....	....	78.7	77.1	....	73.9	71.2	67.1	111.6
16.5	....	....	81.4	79.9	....	....	73.8	69.6	115.0
17.0	....	....	84.2	82.6	....	....	....	72.0	....
17.5	....	....	86.9	....	....	....	....	74.5	....
18.0	....	....	89.6	....	....	....	....	....	....

* Taken from Bruhns, *Deut. Zuckerind.*, **55**, 120 (1930); **58**, 287 (1933), and from Frühling-Spengler, "Anleitung zu Untersuchungen in der Zuckerindustrie," 10th ed., pp. 330–332, 1932. See text, p. 835.

TABLE 31*

Kröber's Table for Determining Pentoses and Pentosans

Furfural Phloroglucide	Furfural	Arabinose	Araban	Xylose	Xylan	Pentose	Pentosan
grams	grams	grams	grams	grams	grams	grams	grams
0.030	0.0182	0.0391	0.0344	0.0324	0.0285	0.0358	0.0315
.031	.0188	.0402	.0354	.0333	.0293	.0368	.0324
.032	.0193	.0413	.0363	.0342	.0301	.0378	.0333
.033	.0198	.0424	.0373	.0352	.0309	.0388	.0341
.034	.0203	.0435	.0383	.0361	.0317	.0398	.0350
.035	.0209	.0446	.0393	.0370	.0326	.0408	.0359
.036	.0214	.0457	.0402	.0379	.0334	.0418	.0368
.037	.0219	.0468	.0412	.0388	.0342	.0428	.0377
.038	.0224	.0479	.0422	.0398	.0350	.0439	.0386
.039	.0229	.0490	.0431	.0407	.0358	.0449	.0395
.040	.0235	.0501	.0441	.0416	.0366	.0459	.0404
.041	.0240	.0512	.0451	.0425	.0374	.0469	.0413
.042	.0245	.0523	.0460	.0434	.0382	.0479	.0422
.043	.0250	.0534	.0470	.0443	.0390	.0489	.0431
.044	.0255	.0545	.0480	.0452	.0398	.0499	.0440
.045	.0260	.0556	.0490	.0462	.0406	.0509	.0448
.046	.0266	.0567	.0499	.0471	.0414	.0519	.0457
.047	.0271	.0578	.0509	.0480	.0422	.0529	.0466
.048	.0276	.0589	.0519	.0489	.0430	.0539	.0475
.049	.0281	.0600	.0528	.0498	.0438	.0549	.0484
.050	.0286	.0611	.0538	.0507	.0446	.0559	.0492
.051	.0292	.0622	.0548	.0516	.0454	.0569	.0501
.052	.0297	.0633	.0557	.0525	.0462	.0579	.0510
.053	.0302	.0644	.0567	.0534	.0470	.0589	.0519
.054	.0307	.0655	.0576	.0543	.0478	.0599	.0528
.055	.0312	.0666	.0586	.0553	.0486	.0610	.0537
.056	.0318	.0677	.0596	.0562	.0494	.0620	.0546
.057	.0323	.0688	.0605	.0571	.0502	.0630	.0555
.058	.0328	.0699	.0615	.0580	.0510	.0640	.0564
.059	.0333	.0710	.0624	.0589	.0518	.0650	.0573
.060	.0338	.0721	.0634	.0598	.0526	.0660	.0581
.061	.0344	.0732	.0644	.0607	.0534	.0670	.0590
.062	.0349	.0743	.0653	.0616	.0542	.0680	.0599
.063	.0354	.0754	.0663	.0626	.0550	.0690	.0608
.064	.0359	.0765	.0673	.0635	.0558	.0700	.0617
.065	.0364	.0776	.0683	.0644	.0567	.0710	.0625
.066	.0370	.0787	.0692	.0653	.0575	.0720	.0634
.067	.0375	.0798	.0702	.0662	.0583	.0730	.0643
.068	.0380	.0809	.0712	.0672	.0591	.0741	.0652
.069	.0385	.0820	.0721	.0681	.0599	.0751	.0661
.070	.0390	.0831	.0731	.0690	.0607	.0761	.0670
.071	.0396	.0842	.0741	.0699	.0615	.0771	.0679
.072	.0401	.0853	.0750	.0708	.0623	.0781	.0688
.073	.0406	.0864	.0760	.0717	.0631	.0791	.0697
.074	.0411	.0875	.0770	.0726	.0639	.0801	.0706

* See text, p. 907. Taken from *J. Landw.*, **48**, 355 (1900); **49**, 7 (1901).

TABLE 31 (*Continued*)

Furfural Phloroglucide	Furfural	Arabinose	Araban	Xylose	Xylan	Pentose	Pentosan
grams	grams	grams	grams	grams	grams	grams	grams
0.075	0.0416	0.0886	0.0780	0.0736	0.0647	0.0811	0.0714
.076	.0422	.0897	.0789	.0745	.0655	.0821	.0722
.077	.0427	.0908	.0799	.0754	.0663	.0831	.0731
.078	.0432	.0919	.0809	.0763	.0671	.0841	.0740
.079	.0437	.0930	.0818	.0772	.0679	.0851	.0749
.080	.0442	.0941	.0828	.0781	.0687	.0861	.0758
.081	.0448	.0952	.0838	.0790	.0695	.0871	.0767
.082	.0453	.0963	.0847	.0799	.0703	.0881	.0776
.083	.0458	.0974	.0857	.0808	.0711	.0891	.0785
.084	.0463	.0985	.0867	.0817	.0719	.0901	.0794
.085	.0468	.0996	.0877	.0827	.0727	.0912	.0803
.086	.0474	.1007	.0886	.0836	.0735	.0922	.0812
.087	.0479	.1018	.0896	.0845	.0743	.0932	.0821
.088	.0484	.1029	.0906	.0854	.0751	.0942	.0830
.089	.0489	.1040	.0915	.0863	.0759	.0952	.0838
.090	.0494	.1051	.0925	.0872	.0767	.0962	.0847
.091	.0499	.1062	.0935	.0881	.0775	.0972	.0856
.092	.0505	.1073	.0944	.0890	.0783	.0982	.0865
.093	.0510	.1084	.0954	.0900	.0791	.0992	.0874
.094	.0515	.1095	.0964	.0909	.0800	.1002	.0883
.095	.0520	.1106	.0974	.0918	.0808	.1012	.0891
.096	.0525	.1117	.0983	.0927	.0816	.1022	.0899
.097	.0531	.1128	.0993	.0936	.0824	.1032	.0908
.098	.0536	.1139	.1003	.0946	.0832	.1043	.0917
.099	.0541	.1150	.1012	.0955	.0840	.1053	.0926
.100	.0546	.1161	.1022	.0964	.0848	.1063	.0935
.101	.0551	.1171	.1032	.0973	.0856	.1073	.0944
.102	.0557	.1182	.1041	.0982	.0864	.1083	.0953
.103	.0562	.1193	.1051	.0991	.0872	.1093	.0962
.104	.0567	.1204	.1060	.1000	.0880	.1103	.0971
.105	.0572	.1215	.1070	.1010	.0888	.1113	.0979
.106	.0577	.1226	.1080	.1019	.0896	.1123	.0988
.107	.0582	.1237	.1089	.1028	.0904	.1133	.0997
.108	.0588	.1248	.1099	.1037	.0912	.1143	.1006
.109	.0593	.1259	.1108	.1046	.0920	.1153	.1015
.110	.0598	.1270	.1118	.1055	.0928	.1163	.1023
.111	.0603	.1281	.1128	.1064	.0936	.1173	.1032
.112	.0608	.1292	.1137	.1073	.0944	.1183	.1041
.113	.0614	.1303	.1147	.1082	.0952	.1193	.1050
.114	.0619	.1314	.1156	.1091	.0960	.1203	.1059
.115	.0624	.1325	.1166	.1101	.0968	.1213	.1067
.116	.0629	.1336	.1176	.1110	.0976	.1223	.1076
.117	.0634	.1347	.1185	.1119	.0984	.1233	.1085
.118	.0640	.1358	.1195	.1128	.0992	.1243	.1094
.119	.0645	.1369	.1204	.1137	.1000	.1253	.1103

TABLE 31 (*Continued*)

Furfural Phloroglucide	Furfural	Arabinose	Araban	Xylose	Xylan	Pentose	Pentosan
grams	grams	grams	grams	grams	grams	grams	grams
0.120	0.0650	0.1380	0.1214	0.1146	0.1008	0.1263	0.1111
.121	.0655	.1391	.1224	.1155	.1016	.1273	.1120
.122	.0660	.1402	.1233	.1164	.1024	.1283	.1129
.123	.0665	.1413	.1243	.1173	.1032	.1293	.1138
.124	.0671	.1424	.1253	.1182	.1040	.1303	.1147
.125	.0676	.1435	.1263	.1192	.1049	.1314	.1156
.126	.0681	.1446	.1272	.1201	.1057	.1324	.1165
.127	.0686	.1457	.1282	.1210	.1065	.1334	.1174
.128	.0691	.1468	.1292	.1219	.1073	.1344	.1183
.129	.0697	.1479	.1301	.1228	.1081	.1354	.1192
.130	.0702	.1490	.1311	.1237	.1089	.1364	.1201
.131	.0707	.1501	.1321	.1246	.1097	.1374	.1210
.132	.0712	.1512	.1330	.1255	.1105	.1384	.1219
.133	.0717	.1523	.1340	.1264	.1113	.1394	.1227
.134	.0723	.1534	.1350	.1273	.1121	.1404	.1236
.135	.0728	.1545	.1360	.1283	.1129	.1414	.1244
.136	.0733	.1556	.1369	.1292	.1137	.1424	.1253
.137	.0738	.1567	.1379	.1301	.1145	.1434	.1262
.138	.0743	.1578	.1389	.1310	.1153	.1444	.1271
.139	.0748	.1589	.1398	.1319	.1161	.1454	.1280
.140	.0754	.1600	.1408	.1328	.1169	.1464	.1288
.141	.0759	.1611	.1418	.1337	.1177	.1474	.1297
.142	.0764	.1622	.1427	.1346	.1185	.1484	.1306
.143	.0769	.1633	.1437	.1355	.1193	.1494	.1315
.144	.0774	.1644	.1447	.1364	.1201	.1504	.1324
.145	.0780	.1655	.1457	.1374	.1209	.1515	.1333
.146	.0785	.1666	.1466	.1383	.1217	.1525	.1342
.147	.0790	.1677	.1476	.1392	.1225	.1535	.1351
.148	.0795	.1688	.1486	.1401	.1233	.1545	.1360
.149	.0800	.1699	.1495	.1410	.1241	.1555	.1369
.150	.0805	.1710	.1505	.1419	.1249	.1565	.1377
.151	.0811	.1721	.1515	.1428	.1257	.1575	.1386
.152	.0816	.1732	.1524	.1437	.1265	.1585	.1395
.153	.0821	.1743	.1534	.1446	.1273	.1595	.1404
.154	.0826	.1754	.1544	.1455	.1281	.1605	.1413
.155	.0831	.1765	.1554	.1465	.1289	.1615	.1421
.156	.0837	.1776	.1563	.1474	.1297	.1625	.1430
.157	.0842	.1787	.1573	.1483	.1305	.1635	.1439
.158	.0847	.1798	.1583	.1492	.1313	.1645	.1448
.159	.0852	.1809	.1592	.1501	.1321	.1655	.1457
.160	.0857	.1820	.1602	.1510	.1329	.1665	.1465
.161	.0863	.1831	.1612	.1519	.1337	.1675	.1474
.162	.0868	.1842	.1621	.1528	.1345	.1685	.1483
.163	.0873	.1853	.1631	.1537	.1353	.1695	.1492
.164	.0878	.1864	.1640	.1546	.1361	.1705	.1501

TABLE 31 (*Continued*)

Furfural Phloroglucide	Furfural	Arabinose	Araban	Xylose	Xylan	Pentose	Pentosan
grams	grams	grams	grams	grams	grams	grams	grams
0.165	0.0883	0.1875	0.1650	0.1556	0.1369	0.1716	0.1510
.166	.0888	.1886	.1660	.1565	.1377	.1726	.1519
.167	.0894	.1897	.1669	.1574	.1385	.1736	.1528
.168	.0899	.1908	.1679	.1583	.1393	.1746	.1537
.169	.0904	.1919	.1688	.1592	.1401	.1756	.1546
.170	.0909	.1930	.1698	.1601	.1409	.1766	.1554
.171	.0914	.1941	.1708	.1610	.1417	.1776	.1563
.172	.0920	.1952	.1717	.1619	.1425	.1786	.1572
.173	.0925	.1963	.1727	.1628	.1433	.1796	.1581
.174	.0930	.1974	.1736	.1637	.1441	.1806	.1590
.175	.0935	.1985	.1746	.1647	.1449	.1816	.1598
.176	.0940	.1996	.1756	.1656	.1457	.1826	.1607
.177	.0946	.2007	.1765	.1665	.1465	.1836	.1616
.178	.0951	.2018	.1775	.1674	.1473	.1846	.1625
.179	.0956	.2029	.1784	.1683	.1481	.1856	.1634
.180	.0961	.2039	.1794	.1692	.1489	.1866	.1642
.181	.0966	.2050	.1804	.1701	.1497	.1876	.1651
.182	.0971	.2061	.1813	.1710	.1505	.1886	.1660
.183	.0977	.2072	.1823	.1719	.1513	.1896	.1669
.184	.0982	.2082	.1832	.1728	.1521	.1906	.1678
.185	.0987	.2093	.1842	.1738	.1529	.1916	.1686
.186	.0992	.2104	.1851	.1747	.1537	.1926	.1695
.187	.0997	.2115	.1861	.1756	.1545	.1936	.1704
.188	.1003	.2126	.1870	.1765	.1553	.1946	.1712
.189	.1008	.2136	.1880	.1774	.1561	.1955	.1721
.190	.1013	.2147	.1889	.1783	.1569	.1965	.1729
.191	.1018	.2158	.1899	.1792	.1577	.1975	.1738
.192	.1023	.2168	.1908	.1801	.1585	.1985	.1747
.193	.1028	.2179	.1918	.1810	.1593	.1995	.1756
.194	.1034	.2190	.1927	.1819	.1601	.2005	.1764
.195	.1039	.2201	.1937	.1829	.1609	.2015	.1773
.196	.1044	.2212	.1946	.1838	.1617	.2025	.1782
.197	.1049	.2222	.1956	.1847	.1625	.2035	.1791
.198	.1054	.2233	.1965	.1856	.1633	.2045	.1800
.199	.1059	.2244	.1975	.1865	.1641	.2055	.1808
.200	.1065	.2255	.1984	.1874	.1649	.2065	.1817
.201	.1070	.2266	.1994	.1883	.1657	.2075	.1826
.202	.1075	.2276	.2003	.1892	.1665	.2085	.1835
.203	.1080	.2287	.2013	.1901	.1673	.2095	.1844
.204	.1085	.2298	.2022	.1910	.1681	.2105	.1853
.205	.1090	.2309	.2032	.1920	.1689	.2115	.1861
.206	.1096	.2320	.2041	.1929	.1697	.2125	.1869
.207	.1101	.2330	.2051	.1938	.1705	.2134	.1878
.208	.1106	.2341	.2060	.1947	.1713	.2144	.1887
.209	.1111	.2352	.2069	.1956	.1721	.2154	.1896

TABLE 31 (*Continued*)

Furfural Phloroglucide	Furfural	Arabinose	Araban	Xylose	Xylan	Pentose	Pentosan
grams	grams	grams	grams	grams	grams	grams	grams
0.210	0.1116	0.2363	0.2079	0.1965	0.1729	0.2164	0.1904
.211	.1121	.2374	.2089	.1975	.1737	.2174	.1913
.212	.1127	.2384	.2098	.1984	.1745	.2184	.1922
.213	.1132	.2395	.2108	.1993	.1753	.2194	.1931
.214	.1137	.2406	.2117	.2002	.1761	.2204	.1940
.215	.1142	.2417	.2127	.2011	.1770	.2214	.1948
.216	.1147	.2428	.2136	.2020	.1778	.2224	.1957
.217	.1152	.2438	.2146	.2029	.1786	.2234	.1966
.218	.1158	.2449	.2155	.2038	.1794	.2244	.1974
.219	.1163	.2460	.2165	.2047	.1802	.2254	.1983
.220	.1168	.2471	.2174	.2057	.1810	.2264	.1992
.221	.1173	.2482	.2184	.2066	.1818	.2274	.2001
.222	.1178	.2492	.2193	.2075	.1826	.2284	.2010
.223	.1183	.2503	.2203	.2084	.1834	.2294	.2019
.224	.1189	.2514	.2212	.2093	.1842	.2304	.2028
.225	.1194	.2525	.2222	.2102	.1850	.2314	.2037
.226	.1199	.2536	.2232	.2111	.1858	.2324	.2046
.227	.1204	.2546	.2241	.2121	.1866	.2334	.2054
.228	.1209	.2557	.2251	.2130	.1874	.2344	.2063
.229	.1214	.2568	.2260	.2139	.1882	.2354	.2072
.230	.1220	.2579	.2270	.2148	.1890	.2364	.2081
.231	.1225	.2590	.2280	.2157	.1898	.2374	.2089
.232	.1230	.2600	.2289	.2166	.1906	.2383	.2097
.233	.1235	.2611	.2299	.2175	.1914	.2393	.2106
.234	.1240	.2622	.2308	.2184	.1922	.2403	.2115
.235	.1245	.2633	.2318	.2193	.1930	.2413	.2124
.236	.1251	.2644	.2327	.2202	.1938	.2423	.2132
.237	.1256	.2654	.2337	.2211	.1946	.2433	.2141
.238	.1261	.2665	.2346	.2220	.1954	.2443	.2150
.239	.1266	.2676	.2356	.2229	.1962	.2453	.2159
.240	.1271	.2687	.2365	.2239	.1970	.2463	.2168
.241	.1276	.2698	.2375	.2248	.1978	.2473	.2176
.242	.1281	.2708	.2384	.2257	.1986	.2483	.2185
.243	.1287	.2719	.2394	.2266	.1994	.2493	.2194
.244	.1292	.2730	.2403	.2275	.2002	.2503	.2203
.245	.1297	.2741	.2413	.2284	.2010	.2513	.2212
.246	.1302	.2752	.2422	.2293	.2018	.2523	.2220
.247	.1307	.2762	.2432	.2302	.2026	.2533	.2229
.248	.1312	.2773	.2441	.2311	.2034	.2543	.2238
.249	.1318	.2784	.2451	.2320	.2042	.2553	.2247
.250	.1323	.2795	.2460	.2330	.2050	.2563	.2256
.251	.1328	.2806	.2470	.2339	.2058	.2573	.2264
.252	.1333	.2816	.2479	.2348	.2066	.2582	.2272
.253	.1338	.2827	.2489	.2357	.2074	.2592	.2281
.254	.1343	.2838	.2498	.2366	.2082	.2602	.2290

TABLE 31 (*Concluded*)

Furfural Phloroglucide	Furfural	Arabinose	Araban	Xylose	Xylan	Pentose	Pentosan
grams	grams	grams	grams	grams	grams	grams	grams
0.255	0.1349	0.2849	0.2508	0.2375	0.2090	0.2612	0.2299
.256	.1354	.2860	.2517	.2384	.2098	.2622	.2307
.257	.1359	.2870	.2526	.2393	.2106	.2632	.2316
.258	.1364	.2881	.2536	.2402	.2114	.2642	.2325
.259	.1369	.2892	.2545	.2411	.2122	.2652	.2334
.260	.1374	.2903	.2555	.2420	.2130	.2662	.2342
.261	.1380	.2914	.2565	.2429	.2138	.2672	.2351
.262	.1385	.2924	.2574	.2438	.2146	.2681	.2359
.263	.1390	.2935	.2584	.2447	.2154	.2691	.2368
.264	.1395	.2946	.2593	.2456	.2162	.2701	.2377
.265	.1400	.2957	.2603	.2465	.2170	.2711	.2385
.266	.1405	.2968	.2612	.2474	.2178	.2721	.2394
.267	.1411	.2978	.2622	.2483	.2186	.2731	.2403
.268	.1416	.2989	.2631	.2492	.2194	.2741	.2412
.269	.1421	.3000	.2641	.2502	.2202	.2751	.2421
.270	.1426	.3011	.2650	.2511	.2210	.2761	.2429
.271	.1431	.3022	.2660	.2520	.2218	.2771	.2438
.272	.1436	.3032	.2669	.2529	.2226	.2781	.2447
.273	.1442	.3043	.2679	.2538	.2234	.2791	.2456
.274	.1447	.3054	.2688	.2547	.2242	.2801	.2465
.275	.1452	.3065	.2698	.2556	.2250	.2811	.2473
.276	.1457	.3076	.2707	.2565	.2258	.2821	.2482
.277	.1462	.3086	.2717	.2574	.2266	.2830	.2490
.278	.1467	.3097	.2726	.2583	.2274	.2840	.2499
.279	.1473	.3108	.2736	.2592	.2282	.2850	.2508
.280	.1478	.3119	.2745	.2602	.2290	.2861	.2517
.281	.1483	.3130	.2755	.2611	.2298	.2871	.2526
.282	.1488	.3140	.2764	.2620	.2306	.2880	.2534
.283	.1493	.3151	.2774	.2629	.2314	.2890	.2543
.284	.1498	.3162	.2783	.2638	.2322	.2900	.2552
.285	.1504	.3173	.2793	.2647	.2330	.2910	.2561
.286	.1509	.3184	.2802	.2656	.2338	.2920	.2570
.287	.1514	.3194	.2812	.2665	.2346	.2930	.2578
.288	.1519	.3205	.2821	.2674	.2354	.2940	.2587
.289	.1524	.3216	.2831	.2683	.2362	.2950	.2596
290	.1529	.3227	.2840	.2693	.2370	.2960	.2605
.291	.1535	.3238	.2850	.2702	.2378	.2970	.2614
.292	.1540	.3248	.2859	.2711	.2386	.2980	.2622
.293	.1545	.3259	.2868	.2720	.2394	.2990	.2631
.294	.1550	.3270	.2878	.2729	.2402	.3000	.2640
.295	.1555	.3281	.2887	.2738	.2410	.3010	.2649
.296	.1560	.3292	.2897	.2747	.2418	.3020	.2658
.297	.1566	.3302	.2906	.2756	2426	.3030	.2666
.298	.1571	.3313	.2916	.2765	2434	.3040	.2675
.299	.1576	.3324	.2925	.2774	.2442	.3050	.2684
.300	.1581	.3335	2935	.2784	.2450	.3060	.2693

TABLE 32*

Tollens, Ellett, and Mayer's Table for Determining Methylpentoses and Methylpentosans

Methylfurfural Phloroglucide	Fucose	Fucosan (Fucose × 0.89)	Rhamnose	Rhamnosan (Rhamnose × 0.8)	Methylpentosan (Average of Fucosan and Rhamnosan)
grams	grams	grams	grams	grams	grams
0.010	0.0260	0.0231	0.0266	0.0213	0.0222
0.011	0.0284	0.0253	0.0279	0.0223	0.0238
0.012	0.0307	0.0274	0.0295	0.0236	0.0255
0.013	0.0331	0.0295	0.0311	0.0249	0.0272
0.014	0.0354	0.0315	0.0327	0.0262	0.0288
0.015	0.0377	0.0336	0.0343	0.0274	0.0305
0.016	0.0400	0.0356	0.0359	0.0287	0.0321
0.017	0.0423	0.0376	0.0375	0.0300	0.0338
0.018	0.0445	0.0396	0.0391	0.0313	0.0354
0.019	0.0467	0.0416	0.0407	0.0326	0.0371
0.020	0.0489	0.0435	0.0423	0.0338	0.0386
0.021	0.0510	0.0454	0.0438	0.0350	0.0402
0.022	0.0532	0.0473	0.0454	0.0363	0.0418
0.023	0.0553	0.0492	0.0469	0.0375	0.0433
0.024	0.0574	0.0511	0.0485	0.0388	0.0449
0.025	0.0594	0.0529	0.0500	0.0400	0.0462
0.026	0.0614	0.0547	0.0516	0.0413	0.0480
0.027	0.0634	0.0565	0.0531	0.0425	0.0495
0.028	0.0654	0.0583	0.0547	0.0438	0.0510
0.029	0.0674	0.0600	0.0562	0.0450	0.0525
0.030	0.0693	0.0617	0.0578	0.0462	0.0539
0.031	0.0712	0.0634	0.0593	0.0474	0.0554
0.032	0.0731	0.0651	0.0609	0.0487	0.0569
0.033	0.0750	0.0668	0.0624	0.0499	0.0584
0.034	0.0768	0.0684	0.0639	0.0511	0.0598
0.035	0.0786	0.0700	0.0655	0.0524	0.0612
0.036	0.0804	0.0716	0.0670	0.0536	0.0626
0.037	0.0822	0.0732	0.0685	0.0548	0.0640
0.038	0.0839	0.0747	0.0700	0.0560	0.0654
0.039	0.0857	0.0764	0.0716	0.0573	0.0668
0.040	0.0874	0.0778	0.0731	0.0585	0.0681
0.041	0.0890	0.0792	0.0747	0.0598	0.0695
0.042	0.0907	0.0807	0.0761	0.0609	0.0708
0.043	0.0923	0.0821	0.0775	0.0620	0.0721
0.044	0.0939	0.0836	0.0790	0.0632	0.0734
0.045	0.0954	0.0850	0.0803	0.0644	0.0747
0.046	0.0970	0.0863	0.0820	0.0656	0.0759
0.047	0.0985	0.0877	0.0835	0.0668	0.0772
0.048	0.1000	0.0890	0.0849	0.0679	0.0785
0.049	0.1015	0.0903	0.0864	0.0691	0.0797
0.050	0.1029	0.0916	0.0879	0.0703	0.0809

* See text, p. 919. Taken from *Z. Ver deut. Zucker-Ind.*, **57**, 620 (1907).

TABLE 33*

Van der Haar's Table for Determining Galactose Alone by the Mucic Acid Method

Mucic Acid	Galactose	Mucic Acid	Galactose	Mucic Acid	Galactose	Mucic Acid	Galactose
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
−4	0	187	260	383.8	520	597	780
+0.8	10	194	270	392.7	530	606	790
5.6	20	201	280	401.6	540	615	800
10.4	30	208	290	410.5	550	623	810
15.2	40	215	300	419.4	560	631	820
20	50	223.1	310	428.3	570	639	830
27	60	231.2	320	437.2	580	647	840
34	70	239.3	330	446.1	590	655	850
41	80	247.4	340	455	600	663	860
48	90	255.5	350	462	610	671	870
55	100	263.6	360	469	620	679	880
64	110	271.7	370	476	630	688	890
73	120	279.8	380	483	640	695	900
82	130	287.9	390	490	650	703.5	910
91	140	296	400	497	660	712	920
100	150	303	410	504	670	720.5	930
108.4	160	310	420	511	680	729	940
116.8	170	317	430	518	690	737.5	950
125.2	180	324	440	525	700	746	960
133.6	190	331	450	534	710	754.5	970
142	200	338	460	543	720	763	980
149.6	210	345	470	552	730	771.5	990
157.2	220	352	480	561	740	780	1000
164.8	230	359	490	570	750	.....	....
172.4	240	366	500	579	760	.....	....
180	250	374.9	510	588	770	.....	....

* Taken from van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide," p. 125. See text, p. 938.

TABLE 34*

Van der Haar's Table for Determining Galactose in the Presence of Other Sugars, by the Mucic Acid Method

Mucic Acid	Galactose	Mucic Acid	Galactose	Mucic Acid	Galactose	Mucic Acid	Galactose
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
−4	0	173	260	376	520	582	780
+2.4	10	181	270	384	530	591	790
8.8	20	189	280	392	540	600	800
15.2	30	197	290	400	550	609	810
21.6	40	205	300	408	560	618	820
28	50	212	310	416	570	627	830
34.9	60	219	320	424	580	636	840
41.8	70	226	330	432	590	645	850
48.7	80	233	340	440	600	654	860
55.6	90	240	350	447	610	663	870
62.5	100	248.8	360	454	620	672	880
70	110	257.6	370	461	630	681	890
77.5	120	266.4	380	468	640	690	900
85	130	275.2	390	475	650	699	910
92.5	140	284	400	483	660	708	920
100	150	292.2	410	491	670	717	930
106.6	160	300.4	420	499	680	726	940
113.2	170	308.6	430	507	690	735	950
119.8	180	316.8	440	515	700	744	960
126.4	190	325	450	523	710	753	970
133	200	332	460	531	720	762	980
139.4	210	339	470	539	730	771	990
145.8	220	346	480	547	740	780	1000
152.2	230	353	490	555	750	...	....
158.6	240	360	500	564	760	...	....
165	250	368	510	573	770	...	....

* Taken from van der Haar, " Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide," p. 126. See text, p. 938.

TABLE 35*

JACKSON AND MATHEWS'S TABLE OF LANE AND EYNON FACTORS FOR ANALYZING MIXTURES OF GLUCOSE AND FRUCTOSE BY A COMBINATION OF THE LANE AND EYNON METHOD WITH THE JACKSON AND MATHEWS METHOD FOR DETERMINING FRUCTOSE

The factor represents the number of milligrams of sugar required to reduce 25 ml. of Soxhlet reagent

$$100 \times \frac{\text{factor}}{\text{titer}} = \text{mg. sugar in 100 ml.}$$

In the last three columns are titer corrections corresponding to experimentally determined factors which differ from the tabulated factors by 1, 2, and 3 units. When the experimental factor is greater than the tabulated, the correction is to be subtracted from the observed titer, and vice versa. This corrected titer is to be used with the tabulated factor.

Titer	Glucose	10 Per Cent Fructose	20 Per Cent Fructose	30 Per Cent Fructose	40 Per Cent Fructose	Invert Sugar	60 Per Cent Fructose	70 Per Cent Fructose	80 Per Cent Fructose	90 Per Cent Fructose	Fructose	Titer Corrections		
												1	2	3
15	120.2	120.9	121.6	122.2	122.9	123.6	124.4	125.1	125.9	126.6	127.4	0.12	0.24	0.37
16	120.2	120.9	121.6	122.2	122.9	123.6	124.4	125.1	125.9	126.6	127.4	.13	.26	.39
17	120.2	120.9	121.6	122.2	122.9	123.6	124.4	125.2	125.9	126.7	127.5	.14	.28	.41
18	120.2	120.9	121.6	122.3	123.0	123.7	124.5	125.2	126.0	126.7	127.5	.15	.30	.44
19	120.3	121.0	121.7	122.3	123.0	123.7	124.5	125.3	126.0	126.8	127.6	.15	.31	.46
20	120.3	121.0	121.7	122.4	123.1	123.8	124.6	125.3	126.1	126.8	127.6	.16	.33	.49
21	120.3	121.0	121.7	122.4	123.1	123.8	124.6	125.4	126.1	126.9	127.7	.17	.34	.51
22	120.4	121.1	121.8	122.5	123.2	123.9	124.7	125.4	126.2	126.9	127.7	.18	.36	.54
23	120.4	121.1	121.8	122.5	123.2	123.9	124.7	125.5	126.2	127.0	127.8	.19	.37	.56
24	120.5	121.2	121.9	122.6	123.3	124.0	124.8	125.5	126.3	127.0	127.8	.20	.39	.59
25	120.5	121.2	121.9	122.6	123.3	124.0	124.8	125.6	126.3	127.1	127.9	.20	.41	.61
26	120.6	121.3	122.0	122.7	123.4	124.1	124.9	125.6	126.4	127.1	127.9	.21	.42	.63
27	120.6	121.3	122.0	122.7	123.4	124.1	124.9	125.7	126.4	127.2	128.0	.22	.44	.66
28	120.7	121.4	122.1	122.8	123.5	124.2	125.0	125.7	126.5	127.2	128.0	.23	.45	.68
29	120.7	121.4	122.1	122.8	123.5	124.2	125.0	125.8	126.5	127.3	128.1	.24	.47	.71

* Taken from *Bur. Standards J. Research*, 8, 439 (1932). See text, p. 975.

TABLE 35 (*Concluded*)

Titer	Glucose	10 Per Cent Fructose	20 Per Cent Fructose	30 Per Cent Fructose	40 Per Cent Fructose	Invert Sugar	60 Per Cent Fructose	70 Per Cent Fructose	80 Per Cent Fructose	90 Per Cent Fructose	Fructose	Titer Corrections		
												1	2	3
30	120.8	121.5	122.2	122.9	123.6	124.3	125.1	125.8	126.6	127.3	128.1	.25	.49	.73
31	120.8	121.5	122.2	122.9	123.6	124.3	125.1	125.8	126.6	127.3	128.1	.25	.50	.76
32	120.8	121.5	122.2	123.0	123.7	124.4	125.2	125.9	126.7	127.4	128.2	.26	.52	.78
33	120.9	121.6	122.3	123.0	123.7	124.4	125.2	125.9	126.7	127.4	128.2	.27	.54	.80
34	120.9	121.6	122.3	123.1	123.8	124.5	125.3	126.0	126.8	127.5	128.3	.28	.55	.83
35	121.0	121.7	122.4	123.1	123.8	124.5	125.3	126.0	126.8	127.5	128.3	.29	.57	.85
36	121.0	121.7	122.4	123.2	123.9	124.6	125.4	126.1	126.9	127.6	128.4	.29	.59	.88
37	121.1	121.8	122.5	123.2	123.9	124.6	125.4	126.1	126.9	127.6	128.4	.30	.60	.90
38	121.2	121.9	122.6	123.3	124.0	124.7	125.5	126.2	127.0	127.7	128.5	.31	.62	.93
39	121.2	121.9	122.6	123.3	124.0	124.7	125.5	126.2	127.0	127.7	128.5	.32	.63	.95
40	121.2	121.9	122.6	123.4	124.1	124.8	125.6	126.3	127.1	127.8	128.6	.33	.65	.98
41	121.3	122.0	122.7	123.4	124.1	124.8	125.6	126.3	127.1	127.8	128.6	.33	.67	1.00
42	121.4	122.1	122.8	123.5	124.2	124.9	125.6	126.4	127.1	127.9	128.6	.34	.68	1.02
43	121.4	122.1	122.8	123.5	124.2	124.9	125.7	126.4	127.2	127.9	128.7	.35	.70	1.05
44	121.5	122.2	122.9	123.6	124.3	125.0	125.7	126.5	127.2	128.0	128.7	.36	.72	1.07
45	121.5	122.2	122.9	123.6	124.3	125.0	125.8	126.5	127.3	128.0	128.8	.37	.73	1.10
46	121.6	122.3	123.0	123.7	124.4	125.1	125.8	126.6	127.3	128.1	128.8	.37	.75	1.12
47	121.6	122.3	123.0	123.7	124.4	125.1	125.9	126.6	127.4	128.1	128.9	.38	.76	1.15
48	121.7	122.4	123.1	123.8	124.5	125.2	125.9	126.7	127.4	128.2	128.9	.39	.78	1.17
49	121.7	122.4	123.1	123.8	124.5	125.2	126.0	126.7	127.5	128.2	129.0	.40	.80	1.20
50	121.8	122.5	123.2	123.9	124.6	125.3	126.0	126.8	127.5	128.3	129.0	.41	.81	1.22

TABLE 36*

JACKSON AND MATHEWS'S TABLE FOR FINDING THE RATIO OF FRUCTOSE TO TOTAL REDUCING SUGARS BY A COMBINATION OF THE LANE AND EYNON METHOD AND THE METHOD OF JACKSON AND MATHEWS FOR DETERMINING FRUCTOSE

$\frac{T \times l}{100}$	$T = 15$	$T = 25$	$T = 35$	$T = 45$	$\frac{T \times l}{100}$	$T = 15$	$T = 25$	$T = 35$	$T = 45$	$\frac{T \times l}{100}$	$T = 15$	$T = 25$	$T = 35$	$T = 45$
11	1.2	1.2	1.1	1.1	34	21.6	21.5	21.4	21.3	57	41.7	41.5	41.2	40.9
12	2.1	2.1	2.0	2.0	35	22.5	22.4	22.3	22.2	58	42.5	42.3	42.1	41.8
13	3.0	3.0	2.9	2.9	36	23.4	23.3	23.2	23.1	59	43.4	43.2	42.9	42.6
14	3.9	3.8	3.8	3.7	37	24.3	24.2	24.0	23.9	60	44.2	44.0	43.8	43.5
15	4.8	4.7	4.7	4.6	38	25.2	25.1	24.9	24.8	61	45.0	44.8	44.6	44.3
16	5.7	5.6	5.6	5.5	39	26.1	26.0	25.8	25.7	62	45.9	45.7	45.5	45.2
17	6.5	6.5	6.4	6.4	40	26.9	26.8	26.6	26.5	63	46.7	46.5	46.3	46.0
18	7.4	7.4	7.3	7.3	41	27.8	27.7	27.5	27.4	64	47.6	47.4	47.2	46.9
19	8.3	8.2	8.2	8.1	42	28.7	28.6	28.4	28.3	65	48.4	48.2	48.0	47.7
20	9.2	9.1	9.1	9.0	43	29.5	29.4	29.2	29.1	66	49.3	49.0	48.8	48.5
21	10.1	10.0	10.0	9.9	44	30.4	30.3	30.1	30.0	67	50.1	49.9	49.7	49.4
22	11.0	10.9	10.9	10.8	45	31.3	31.2	31.0	30.8	68	51.0	50.8	50.6	50.2
23	11.9	11.8	11.8	11.7	46	32.2	32.0	31.9	31.7	69	51.8	51.6	51.4	51.1
24	12.8	12.7	12.6	12.5	47	33.0	32.9	32.7	32.5	70	52.7	52.5	52.2	51.9
25	13.7	13.6	13.5	13.4	48	33.9	33.8	33.6	33.4	71	53.5	53.3	53.1	52.8
26	14.5	14.4	14.3	14.2	49	34.8	34.6	34.4	34.2	72	54.3	54.1	53.9	53.6
27	15.4	15.3	15.2	15.1	50	35.6	35.4	35.2	35.0	73	55.2	55.0	54.7	54.4
28	16.3	16.2	16.1	16.0	51	36.5	36.3	36.1	35.9	74	56.0	55.8	55.6	55.3
29	17.2	17.1	17.0	16.9	52	37.3	37.2	36.9	36.7	75	56.8	56.6	56.4	56.1
30	18.1	18.0	17.9	17.8	53	38.2	38.0	37.8	37.6	76	57.7	57.5	57.2	56.9
31	19.0	18.9	18.7	18.6	54	39.1	38.9	38.6	38.4	77	58.5	58.3	58.1	57.8
32	19.9	19.8	19.6	19.5	55	39.9	39.7	39.5	39.3	78	59.4	59.2	58.9	58.6
33	20.8	20.6	20.5	20.4	56	40.8	40.6	40.3	40.1	79	60.2	60.0	59.8	59.5

* Taken from *Bur. Standards J. Research*, 8, 444 (1932). See text, p. 975.

TABLE 36 (*Concluded*)

$\frac{T \times l}{100}$	$T = 15$	$T = 25$	$T = 35$	$T = 45$	$\frac{T \times l}{100}$	$T = 15$	$T = 25$	$T = 35$	$T = 45$	$\frac{T \times l}{100}$	$T = 15$	$T = 25$	$T = 35$	$T = 45$
80	61.1	60.9	60.6	60.3	96	74.3	74.2	73.9	73.6	112	87.5	87.2	86.8	86.5
81	61.9	61.7	61.4	61.1	97	75.2	75.0	74.7	74.4	113	88.3	88.0	87.6	87.3
82	62.8	62.5	62.3	62.0	98	76.0	75.8	75.6	75.3	114	89.1	88.8	88.4	88.1
83	63.6	63.3	63.1	62.8	99	76.8	76.6	76.4	76.1	115	90.0	89.6	89.3	88.9
84	64.4	64.2	63.9	63.6	100	77.6	77.4	77.2	76.9	116	90.8	90.4	90.1	89.7
85	65.2	65.0	64.8	64.5	101	78.5	78.2	78.0	77.7	117	91.6	91.3	91.0	90.6
86	66.0	65.8	65.6	65.3	102	79.3	79.0	78.8	78.5	118	92.4	92.1	91.8	91.4
87	66.9	66.7	66.4	66.1	103	80.1	79.9	79.6	79.3	119	93.2	92.9	92.6	92.2
88	67.7	67.5	67.2	66.9	104	81.0	80.7	80.4	80.1	120	94.0	93.7	93.4	93.0
89	68.5	68.3	68.1	67.8	105	81.8	81.5	81.2	80.9	121	94.8	94.5	94.2	93.8
90	69.3	69.1	68.9	68.6	106	82.6	82.3	82.0	81.7	122	95.6	95.3	95.0	94.6
91	70.2	70.0	69.7	69.4	107	83.5	83.2	82.8	82.5	123	96.4	96.1	95.8	95.4
92	71.0	70.8	70.6	70.3	108	84.3	84.0	83.6	83.3	124	97.3	97.0	96.6	96.2
93	71.9	71.7	71.4	71.1	109	85.1	84.8	84.4	84.1	125	98.1	97.8	97.4	97.0
94	72.7	72.5	72.3	72.0	110	85.9	85.6	85.2	84.9	126	98.9	98.6	98.2	97.8
95	73.5	73.3	73.1	72.8	111	86.7	86.4	86.0	85.7	127	99.7	99.4	99.0	98.6

TABLE 37*

MATHEWS'S TABLE FOR FINDING THE RATIO OF FRUCTOSE TO TOTAL REDUCING SUGARS BY A COMBINATION OF THE POLARIZATION WITH THE METHOD OF LANE AND EYNON

The table gives the per cent ratio (R) of fructose to total reducing sugar calculated from the Lane and Eynon titration and the direct polarization at 20° C. P is the polarization in ° S; T is the corrected Lane and Eynon titer; D is the number of volumes to which one volume of the solution polarized was diluted for the titration; and f is the factor used for correcting the per cent ratio found in the table. This correction is given by

$$\frac{f \times D}{T}$$

and is to be added algebraically

The table can be used when the polarization is made at a temperature other than 20° C. by using the temperature coefficient, $\Delta R/\Delta t°$. When the temperature is $t°$, the correction is $\frac{\Delta R}{\Delta t°}(t° - 20°)$, and is to be added algebraically to the ratio.

$\frac{P \cdot T}{D}$	$T=15$	$T=25$	$T=35$	$T=45$	f	$\frac{\Delta R}{\Delta t°}$	$\frac{P \cdot T}{D}$	$T=15$	$T=25$	$T=35$	$T=45$	f	$\frac{\Delta R}{\Delta t°}$	$\frac{P \cdot T}{D}$	$T=15$	$T=25$	$T=35$	$T=45$	f	$\frac{\Delta R}{\Delta t°}$
37	−0.6	−0.4	−0.3	−0.2	0.29	0	22	13.8	13.9	14.0	14.1	0.12	0.06	7	28.2	28.3	28.3	28.3	−0.06	0.12
36	+0.4	+0.5	+0.7	+0.8	.28	0	21	14.8	14.9	15.0	15.0	.11	.07	6	29.2	29.2	29.3	29.3	−.07	.13
35	1.4	1.5	1.6	1.7	.27	0.01	20	15.8	15.8	15.9	16.0	.09	.07	5	30.2	30.2	30.2	30.2	−.08	.13
34	2.3	2.4	2.6	2.7	.26	.01	19	16.7	16.8	16.9	16.9	.08	.07	4	31.1	31.1	31.2	31.2	−.09	.14
33	3.3	3.4	3.5	3.6	.24	.02	18	17.7	17.7	17.8	17.9	.07	.08	3	32.1	32.1	32.1	32.1	−.10	.14
32	4.2	4.4	4.5	4.6	.23	.02	17	18.6	18.7	18.8	18.8	.06	.08	2	33.0	33.0	33.1	33.1	−.11	.14
31	5.2	5.3	5.4	5.5	.22	.02	16	19.6	19.6	19.7	19.8	.05	.09	1	34.0	34.0	34.0	34.0	−.13	.15
30	6.2	6.3	6.4	6.5	.21	.03	15	20.6	20.6	20.7	20.7	.04	.09	0	35.0	35.0	35.0	35.0	−.14	.15
29	7.1	7.2	7.3	7.4	.20	.03	14	21.5	21.6	21.6	21.7	.02	.10	−1	35.9	35.9	35.9	35.9	−.15	.16
28	8.1	8.2	8.3	8.4	.19	.04	13	22.5	22.5	22.6	22.6	.01	.10	−2	36.9	36.9	36.9	36.9	−.16	.16
27	9.0	9.1	9.2	9.3	.18	.04	12	23.4	23.5	23.5	23.6	.00	.10	−3	37.8	37.8	37.8	37.8	−.17	.17
26	10 0	10.1	10.2	10.3	.16	.04	11	24.4	24.4	24.5	24.5	−.01	.11	−4	38.8	38.8	38.8	38.8	−.18	.17
25	11.0	11 1	11.1	11.2	.15	.05	10	25.4	25.4	25.4	25.5	−.02	.11	−5	39.8	39.8	39.7	39.7	−.20	.17
24	11 9	12.0	12.1	12 2	.14	.05	9	26.3	26.4	26.4	26.4	−.03	.12	−6	40.7	40.7	40.7	40.7	−.21	.18
23	12.9	13.0	13.1	13.1	.13	.06	8	27.3	27.3	27.3	27.4	−.05	.12	−7	41.7	41.7	41.6	41.6	−.22	.18

* Taken from *Bur. Standards J. Research*, 8, 442 (1932). See text, p. 984.

TABLE 37 — *Concluded*

$\frac{P.T}{D}$	$T=15$	$T=25$	$T=35$	$T=45$	f	$\frac{\Delta R}{\Delta t^\circ}$	$\frac{P.T}{D}$	$T=15$	$T=25$	$T=35$	$T=45$	f	$\frac{\Delta R}{\Delta t^\circ}$	$\frac{P.T}{D}$	$T=15$	$T=25$	$T=35$	$T=45$	f	$\frac{\Delta R}{\Delta t^\circ}$
−8	42.6	42.6	42.6	42.6	−0.23	0.19	−29	62.8	62.7	62.6	62.5	−0.47	0.27	−50	83.0	82.8	82.6	82.5	−0.72	0.36
−9	43.6	43.6	43.6	43.5	−.24	.19	−30	63.8	63.7	63.6	63.5	−.49	.28	−51	83.9	83.7	83.6	83.4	−.73	.37
−10	44.6	44.5	44.5	44.5	−.25	.19	−31	64.7	64.6	64.5	64.4	−.50	.28	−52	84.9	84.7	84.5	84.4	−.74	.37
−11	45.5	45.5	45.5	45.4	−.27	.20	−32	65.7	65.6	65.5	65.4	−.51	.29	−53	85.8	85.7	85.5	85.3	−.75	.37
−12	46.5	46.4	46.4	46.4	−.28	.20	−33	66.6	66.5	66.4	66.3	−.52	.29	−54	86.8	86.6	86.4	86.3	−.76	.38
−13	47.4	47.4	47.4	47.3	−.29	.21	−34	67.6	67.5	67.4	67.3	−.53	.29	−55	87.8	87.6	87.4	87.2	−.78	.38
−14	48.4	48.4	48.3	48.3	−.30	.21	−35	68.6	68.4	68.3	68.2	−.54	.30	−56	88.7	88.5	88.3	88.2	−.79	.39
−15	49.4	49.3	49.3	49.2	−.31	.22	−36	69.5	69.4	69.3	69.2	−.56	.30	−57	89.7	89.5	89.3	89.1	−.80	.39
−16	50.3	50.3	50.2	50.2	−.32	.22	−37	70.5	70.3	70.2	70.1	−.57	.31	−58	90.6	90.4	90.3	90.1	−.81	.39
−17	51.3	51.2	51.2	51.1	−.34	.22	−38	71.4	71.3	71.2	71.1	−.58	.31	−59	91.6	91.4	91.2	91.0	−.82	.40
−18	52.2	52.2	52.1	52.1	−.35	.23	−39	72.4	72.3	72.4	72.0	−.59	.32	−60	92.6	92.3	92.2	92.0	−.83	.40
−19	53.2	53.1	53.1	53.0	−.36	.23	−40	73.4	73.2	73.1	73.0	−.60	.32	−61	93.5	93.3	93.1	92.9	−.85	.41
−20	54.2	54.1	54.0	54.0	−.37	.24	−41	74.3	74.2	74.1	73.9	−.61	.32	−62	94.5	94.3	94.1	93.9	−.86	.41
−21	55.1	55.0	55.0	54.9	−.38	.24	−42	75.3	75.1	75.0	74.9	−.63	.33	−63	95.4	95.2	95.0	94.8	−.87	.42
−22	56.1	56.0	56.0	55.9	−.39	.24	−43	76.2	76.1	76.0	75.8	−.64	.33	−64	96.4	96.2	96.0	95.8	−.88	.42
−23	57.0	57.0	56.9	56.8	−.40	.25	−44	77.2	77.0	76.9	76.8	−.65	.34	−65	97.4	97.1	96.9	96.7	−.89	.42
−24	58.0	57.9	57.9	57.8	−.42	.25	−45	78.2	78.0	77.9	77.7	−.66	.34	−66	98.3	98.1	97.9	97.7	−.90	.43
−25	59.0	58.9	58.8	58.7	−.43	.26	−46	79.1	79.0	78.8	78.7	−.67	.34	−67	99.3	99.0	98.8	98.6	−.92	.43
−26	59.9	59.8	59.8	59.7	−.44	.26	−47	80.1	79.9	79.8	79.6	−.68	.35	−68	100.2	100.0	99.8	99.6	−.93	.44
−27	60.9	60.8	60.7	60.6	−.45	.27	−48	81.0	80.9	80.7	80.6	−.69	.35	−69	101.2	101.0	100.7	100.5	−.94	.44
−28	61.8	61.7	61.7	61.6	−.46	.27	−49	82.0	81.8	81.7	81.5	−.71	.36	−70	102.2	101.9	101.7	101.5	−.95	.44

TABLE 38*

Zerban's Table of Lane and Eynon Factors and Reducing Ratios for Determining Glucose and Fructose in Raw Sugars

A. 10 Grams Sucrose				B. 25 Grams Sucrose			
Titer	Lane and Eynon Factor	Reducing Ratio a	$a - 0.0806$	Titer	Lane and Eynon Factor	Reducing Ratio a	$a - 0.0806$
15	48.1	1.0434	0.9628	15	44.7	1.0419	0.9613
16	48.0	1.0430	0.9624	16	44.6	1.0396	0.9590
17	47.9	1.0426	0.9620	17	44.4	1.0375	0.9569
18	47.9	1.0422	0.9616	18	44.3	1.0356	0.9550
19	47.8	1.0418	0.9612	19	44.1	1.0339	0.9533
20	47.7	1.0414	0.9608	20	44.0	1.0324	0.9518
21	47.6	1.0410	0.9604	21	43.9	1.0311	0.9505
22	47.5	1.0406	0.9600	22	43.8	1.0300	0.9494
23	47.5	1.0402	0.9596	23	43.7	1.0290	0.9484
24	47.4	1.0398	0.9592	24	43.6	1.0281	0.9475
25	47.3	1.0394	0.9588	25	43.5	1.0273	0.9467
26	47.2	1.0390	0.9584	26	43.4	1.0266	0.9460
27	47.1	1.0386	0.9580	27	43.3	1.0260	0.9454
28	47.1	1.0382	0.9576	28	43.2	1.0255	0.9449
29	47.0	1.0378	0.9572	29	43.1	1.0251	0.9445
30	46.9	1.0374	0.9568	30	43.0	1.0248	0.9442
31	46.8	1.0370	0.9564	31	42.9	1.0247	0.9441
32	46.8	1.0366	0.9560	32	42.9	1.0247	0.9441
33	46.7	1.0362	0.9556	33	42.8	1.0246	0.9440
34	46.7	1.0358	0.9552	34	42.7	1.0246	0.9440
35	46.6	1.0354	0.9548	35	42.6	1.0246	0.9440
36	46.6	1.0350	0.9544	36	42.6	1.0245	0.9439
37	46.5	1.0346	0.9540	37	42.5	1.0245	0.9439
38	46.5	1.0342	0.9536	38	42.5	1.0245	0.9439
39	46.4	1.0338	0.9532	39	42.4	1.0245	0.9439
40	46.4	1.0334	0.9528	40	42.3	1.0245	0.9439
41	46.4	1.0330	0.9524	41	42.2	1.0244	0.9438
42	46.3	1.0326	0.9520	42	42.1	1.0244	0.9438
43	46.3	1.0322	0.9516	43	42.1	1.0244	0.9438
44	46.2	1.0318	0.9512	44	42.0	1.0244	0.9438
45	46.2	1.0314	0.9508	45	41.9	1.0244	0.9438
46	46.2	1.0313	0.9507	46	41.8	1.0243	0.9437
47	46.2	1.0312	0.9506	47	41.7	1.0243	0.9437
48	46.1	1.0312	0.9506	48	41.7	1.0243	0.9437
49	46.1	1.0311	0.9505	49	41.6	1.0243	0.9437
50	46.1	1.0311	0.9505	50	41.5	1.0243	0.9437

* See text, p. 994. Taken from *Ind. Eng. Chem., Anal. Ed.*, **8**, 322 (1936).

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